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BULLETIN OF

EXPERIMENTAL BIOLOGY AND MEDICINE

БЮЛЛЕТЕНЬ ЭКСПЕРИМЕНТАЛЬНОЙ БИОЛОГИИ И МЕДИЦИНЫ

BYULLETEN' ÉKSPERIMENTAL'NOI BIOLOGII I MEDITSINY

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BULLETIN OF

EXPERIMENTAL BIOLOGY AND MEDICINE

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BULLETIN OF EXPERIMENTAL BIOLOGY AND MEDICINE

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CONTENTS

	PAGE	RUSS. PAGE
PHYSIOLOGY		
Electrophysiological Characteristics of the Interoceptive Reflex Arc. Communication II. Characteristics of the Regional Vascular Reflexes Arising During Stimulation of the Mechanoreceptors of the Urinary Bladder. T. S. Lagutina.	1	3
The Effect of Amino Acids on Unconditioned Interoceptive Reflexes. Communication I. The Relationship of Center to Periphery in the Mechanism of Interoceptive Reflexes. L. E. Kramarenko	7	9
PATHOLOGICAL PHYSIOLOGY AND GENERAL PATHOLOGY		
The Mechanism of Excretion of Dysentery Antigen by the Kidneys. M. I. Undritsov	12	15
The Accumulation of Sulfathiazole in Inflammatory Foci of the Skin in Animals While Asleep and Awake. M. Ya. Gerasimov	17	19
BIOCHEMISTRY AND BIOPHYSICS		
The Influence of the Adrenocorticotropic Hormone of the Pituitary Gland on Certain Aspects of Lipid, Carbohydrate and Nitrogen Metabolism in Dogs. G. T. Pavlov	21	24
Does Thiamin Dehydrogenase Exist? S. É. Shnol'	26	29
The Ability of the Body to Retain an Indifferent Gas in a State of Supersaturation. Communication I. The Relationship Between the Permissible Supersaturation and Certain Biophysical and Biochemical Indices in the Blood and the Hydrophilia of the Skin. G. M. Zarakovskii	29	31
The Combined Action of the Spleen and Other Organs. Communication II. The Rate of Renewal of Proteins, The Synthesis of Urea and the Content of the Antianemic Factor in the Liver of White Rats with Experimental Splenopathy. L. I. Geller.	34	35
PHARMACOLOGY		
Biochemical Methods of Removal of the Blocking Action of Methylene Blue on the Vagus Nerve. Kh. S. Koshtoyants and N. E. Babskaya.	38	39
A Reflex Component of the Mechanism of Production of Blood Changes by Chlortetracycline (Biomycin). A. V. Loginov and A. M. Dumova	42	43
MICROBIOLOGY AND IMMUNITY		
Cultivation of Toxoplasma Gundi in Tissue Cultures. G. T. Akunshina	47	48

(continued)

CONTENTS (continued)

	PAGE	RUSS. PAGE
ONCOLOGY		
Prolonged Heterogenic Transplantation of Mouse Tumors in Rats. Communication II. A Study of the Antigenic Properties of Cells of Ehrilich's Ascitic Mouse Carcinoma During Heterotransplantation by Means of the Complement Fixation Reaction. L. I. Podoplelov	51	52
The Action of a Specific Immune Serum on the Development of M-1 Sarcoma in Rats. Z. I. Rovnova	56	56
Some Peculiarities of Metastasis Formation by Experimental Tumors. M. S. Lomakin	62	62
The Effect of Hypothermia on Metastasis of Brown-Pearce Tumors. E. V. Mandrik	66	67
Some Properties of Human "Leukemic Factor" When Cultivated on the Embroyonic Membranes of Chicks. V. M. Bergol'ts	71	71
EXPERIMENTAL BIOLOGY		
Tissue Cell Changes in Human Lymphatic Gland Explantates. N. M. Chistova	76	75
Histological Investigation of the Results of Tendon Repair in Rabbits Using Various Plastic Materials. A. I. Artamonova	83	79
The Action of Immune Sera on the Growth of the Heart and Crystalline Lens of Chick Embryos. O. E. Vyazov and Yu. S. Bocharov	87	83
A Comparative Study of the Antigenic Properties of the Crystalline Lens in Vertebrates and Inverterbrates. B. V. Konyukhov	91	86
Reestablishment of Cell Division After Reactive Inhibition by Depression of the Sympatheticoadrenal System. V. V. Kozlov	97	92
The Mitotic Activity of the Epidermis of the Limb in Rats After Denervation. A. N. Kulagin	103	97
A Study of the Effect of Novocain Blockade on Intact Muscle by Means of Vital Staining. N. I. Grebenskaya	107	101
MORPHOLOGY AND PATHOMORPHOLOGY		
Morphological Changes in the Heart, Lungs and Brain After Experimental Cavopulmonary Anastomosis. T. M. Darbinyan and L. D. Krymskii	111	105
Changes in the Glycogen Content of the Nervous System and Muscles in Animals Infected by Vibrion Septique. E. A. Skvirskaya	115	109
METHODS		
Continuous Recording of the Systolic Blood Pressure by a Bloodless Method Using a Simple Tracing System. V. V. Orlov	120	114
A Bloodless Method of Determining the Systolic Arterial Pressure and the Degree of Vasodilation and Vasoconstriction in White Rats. I. S. Kanfor	125	118
The Use of Lead Nitrate for Detection of the Intracellular Apparatus Reticular. V. F. Kondalenko	129	121
Some Practical Observations on the Bielschowsky – Gros Impregnation Method. T. I. Bogdanova	132	122

PHYSIOLOGY

ELECTROPHYSIOLOGICAL CHARACTERISTICS OF THE INTEROCEPTIVE REFLEX ARC

COMMUNICATION II. CHARACTERISTICS OF THE REGIONAL VASCULAR REFLEXES ARISING DURING STIMULATION OF THE MECHANORECEPTORS OF THE URINARY BLADDER

T. S. Lagutina

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(Received August 17, 1957. Presented by Active Member of the AMN SSSR V. N. Chernigovskii)

In previous communications [1, 2] we made an attempt to compare the true and composite reflexes arising during stimulation of the mechanoreceptors of the urinary bladder. An example of a true reflex, changes in the tone of the ureter were studied, and of a composite reflex — changes in the general arterial pressure.

It was shown that as the strength of stimulation of the mechanoreceptors of the urinary bladder increased, there was a corresponding increase in the intensity of afferent impulses in the pelvic nerve, whereas the efferent impulses to the ureter, like the tone of the ureter, are altered in different ways depending on the strength of stimulation: weak stimuli (3 - 25 mm of mercury) cause an increase in the efferent impulses to the ureter registered in the central end of the pudendal nerve and contraction of that organ. Stimuli of moderate strength (25 - 30 mm of mercury) sometimes cause alternate periods of intensification and weakening of the efferent impulses and rhythmic contraction and relaxation of the ureter. Stronger stimuli (over 25 mm of mercury) cause inhibition of efferent impulses and relaxation of the ureter. Only still greater intensity of stimulation causes reflex changes in the general arterial pressure, i.e. appearance of a composite reflex.

The aim of the present work, which dealt with the interrelation between the true and composite reflexes bringing about the complex reaction of the blood pressure, was to trace the movement within the central nervous system of the process of excitation caused by stimulation of the mechanoreceptors of the urinary bladder. For this purpose we attempted to determine the order in which the following vessels became involved in the reaction — the vessels of the urinary bladder whose mechanoreceptors were being stimulated, the vessels of the kidney as a region functionally connected with the stimulated interoceptive zone, and the vessels of the skin of the hind limb and head as regions anatomically and functionally unconnected with the stimulated zone — and to compare these changes in the individual vascular regions with the reaction of the general arterial pressure.

The majority of present-day workers [5, 6, 7, 8 and others] consider that the reflex regulation of blood vessels is carried out by sympathetic fibers by way of changes in tonic efferent impulsation. These impulses were recorded by several authors [3, 4, 9], who called them "slow" tonic impulses and they either have a constant rhythm or they change synchronously with respiration or with the rhythm of the heart and they differ in different nerves only in frequency or amplitude. These authors consider that the main function of the sympathetic system is to maintain the tone of the blood vessels, i.e. to maintain a constant vasoconstrictor action. However, certain authors [3] could not detect any form of change in these impulses during stimulation of different internal organs.

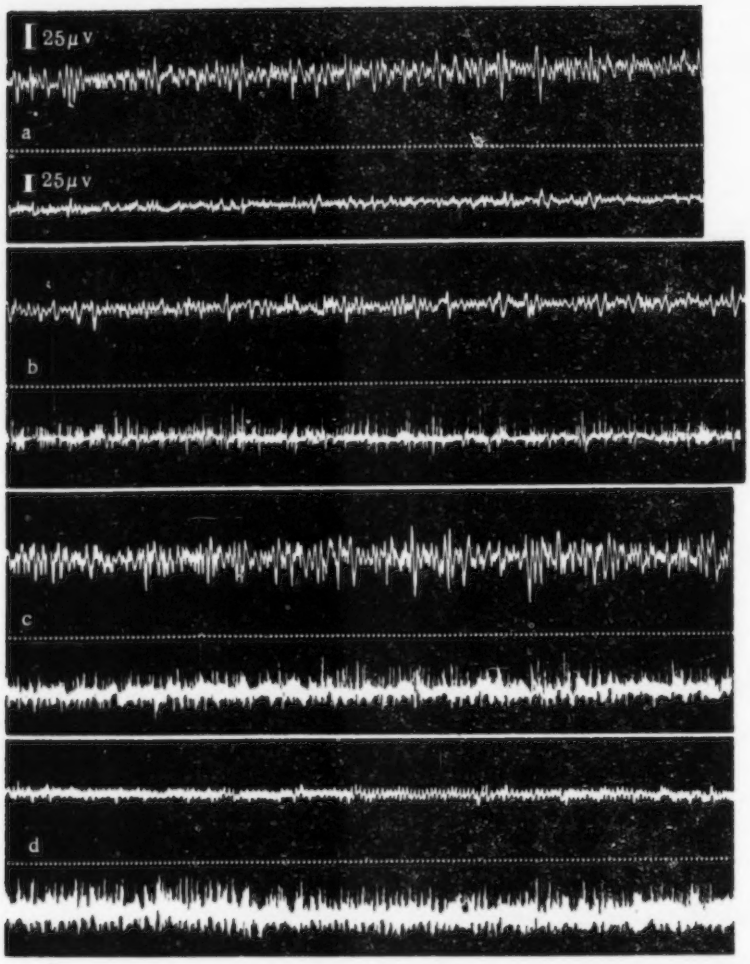

Fig. 1. Reflex changes in the efferent impulsation in the hypogastric nerve and the magnitude of the afferent impulsation in the pelvic nerve during stimulation of varying intensity of the mechanoreceptors of the urinary bladder.
a) Initial efferent impulsation with a rhythm of 68 per sec, amplitude 29 μv. No afferent impulsation present under these conditions (before stimulation); b) pressure inside the urinary bladder increased to 22 mm of mercury. Efferent impulsation with a rhythm of 40 per sec, amplitude 21 μv. Afferent impulsation 130 per sec; c) pressure inside the urinary bladder increased to 56 mm of mercury. Efferent impulsation with a rhythm of 80 per sec, amplitude 37 μv. Afferent impulsation 250 per sec; d) pressure inside the urinary bladder increased to 74 mm of mercury. Efferent impulsation with a rhythm of 20 per sec, amplitude 10 μv. Afferent impulsation 300 per sec. Inscriptions on the oscillograms from above down: action potentials of the central segment of the hypogastric nerve, time marker 0.01 sec, action potentials of the peripheral segment of the pelvic nerve.

EXPERIMENTAL METHOD

In acute experiments on 52 cats (under urethane anesthesia) the arterial pressure in the carotid artery and the pressure inside the urinary bladder were recorded by a mercury manometer. Meanwhile recordings were made on a cathode-ray oscillograph of the impulses in the peripheral end of a branch of the pelvic nerve (afferent

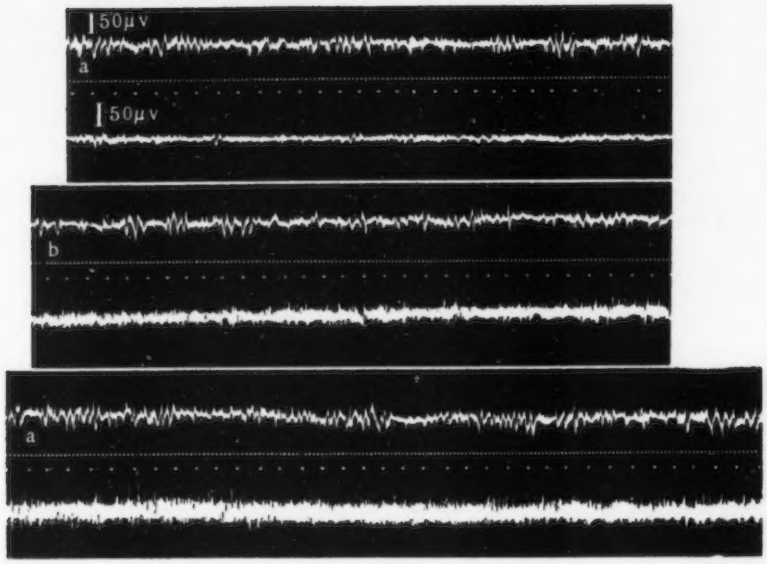

Fig. 2. Reflex changes in the efferent impulsation in the renal nerve and the magnitude of the afferent impulsation in the pelvic nerve during stimulation of varying intensity of the mechanoreceptors of the urinary bladder.
a) Initial efferent impulsation with a rhythm of 45 per sec, amplitude 58 μv. Afferent impulsation in the pelvic nerve absent under these conditions (before stimulation); b) pressure inside the urinary bladder increased to 28 mm of mercury. Efferent impulsation with a rhythm of 45 per sec, amplitude 73 μv. Afferent impulsation 300 per sec. Inscriptions on the oscillograms from above down: action potentials of the central end of the renal nerve, time marker 0.01 sec, time marker 0.05 sec, action potentials of the peripheral end of the pelvic nerve.

impulsation from the mechanoreceptors of the urinary bladder) and the impulses in the central ends of one of the following four nerves: hypogastric, renal, saphenous and the efferent fibers of the cervical sympathetic nerve. In recording the impulses we made use of immersible electrodes as suggested by P. A. Kiselev.

Since the majority of the nerves investigated are mixed, to make sure that the impulses recorded are, in fact, concerned with the regulation of vascular tone, a special series of experiments was carried out in which the vessels of the skin and kidney were perfused. In these experiments the reaction of the vessels were judged by the number of drops flowing from the vein. An increase in the rate of flow of drops indicated vasodilatation, a reduction — vasoconstriction.

EXPERIMENTAL RESULTS

The efferent impulses to the vessels of the urinary bladder were recorded in the central end of the hypogastric nerve, for it is known from morphological investigations [10] that it is in this nerve that the efferent nerve fibers pass to the vessels of the urinary bladder.

In the absence of stimulation of the mechanoreceptors of the urinary bladder a "slow" stream of impulses is observed in the nerve (Fig. 1, a). Weak stimulation of the mechanoreceptors of the urinary bladder (4 - 22 mm of mercury) leads to inhibition of the efferent impulses in the hypogastric nerve (Fig. 1, b). This inhibition develops after a relatively long latent period (from 1 to 4 sec) and reaches its maximum gradually without any concomitant rise of arterial pressure.

The range of variation of intensity of stimulation within which inhibition of efferent impulsation arises in the hypogastric nerve was very small in each experiment. Further increase in the intensity of stimulation of the

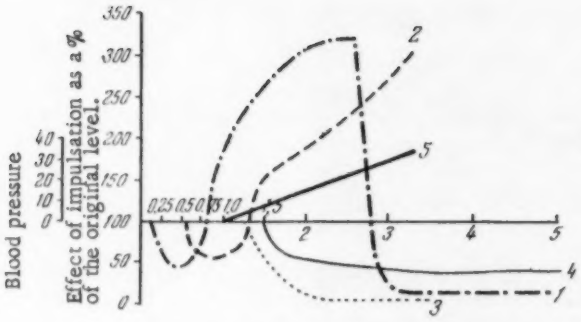

Fig. 3. Relationship between the changes of efferent impulsation in different nerves and the magnitude of the reflex rise in the general blood pressure and the intensity of stimulation of the mechanoreceptors of the urinary bladder.
Along the horizontal axis are represented the strengths of stimulation of the mechanoreceptors of the urinary bladder in relative units. One unit is taken to be the strength of stimulation causing threshold changes in the general blood pressure. Along the vertical axis are represented the changes in the general blood pressure in mm of mercury and changes in the efferent impulsation in different nerves as percentages of their original value. The initial level is taken as 100%. For an explanation, see text. 1) Efferent impulsation in the hypogastric nerve; 2) the same in the renal nerve; 3) the same in the saphenous nerve; 4) the same in the cervical sympathetic trunk; 5) changes in the general arterial pressure.

mechanoreceptors of the urinary bladder then causes strengthening of the efferent impulses in the central end of the hypogastric nerve (Fig. 1, c).

This strengthening of impulsation has a short latent period (0.3 - 1.5 sec) and reaches its maximum quite quickly. The range of intensity of stimulation of the mechanoreceptors of the urinary bladder within which strengthening of the efferent impulsation arises in the hypogastric nerve is very great and varies between 10 and 80 mm of mercury.

It is important to mention that strengthening of the efferent impulsation arises during stimulation of an intensity still inadequate to cause reflex changes in the blood pressure and only after further strengthening does it cause an increase in the blood pressure.

If, however, the pressure in the urinary bladder is increased still further, inhibition of the efferent impulses in the hypogastric nerve develops (Fig. 1, d). This inhibition is characterized by a shorter latent period (0.3 - 1.5 sec) than the inhibition observed by stimulation of low intensity. In all cases the intensity of afferent impulsation in the pelvic nerve increases in proportion to the strength of stimulation (see Fig. 1, a, b, c, d).

We now turn to the analysis of the changes in the efferent impulses in the renal nerves which, in the absence of stimulation of the mechanreceptors of the urinary bladder, consisted of a series of "slow" potentials (Fig. 2, a). Only the first two stages are demonstrated by the changes in the efferent impulsation in the renal nerves: inhibition and strengthening, and furthermore for each of these stages to appear it was necessary to apply more intensive stimulation of the mechanoreceptors of the urinary bladder than for the appearance of the corresponding stages in the nerves of the urinary bladder. The first stage — the stage of inhibition — arose during stimulation at a strength of 15 - 35 mm of mercury (Fig. 2, b). At the time of appearance of a reflex rise of blood pressure, some degree of inhibition of the efferent impulsation to the kidney could still be observed.

Further increase in the intensity of stimulation caused strengthening of the impulses (Fig. 2, c) which lasted as long as the intensity of stimulation of the mechanoreceptors of the urinary bladder continued to increase.

We did not observe the development of a stage of inhibition of the impulses in the renal nerves on further strengthening the stimulation of the mechanoreceptors of the urinary bladder.

From recordings of the efferent impulses in the nerves supplying the skin of the hind limb and in the cervical sympathetic nerve we were able to find only the first stage of changes of impulsation and in the cutaneous nerves this stage appeared at strengths of stimulation (25 mm of mercury and higher) slightly greater than those which caused reflex changes in the arterial pressure.

In the cervical sympathetic nerve inhibition of impulsation developed at still higher strengths of stimulation (28 mm of mercury and higher).

In the special experiments with perfusion of the skin and kidney, which were mentioned above, it could be demonstrated that the efferent impulses recorded in the nerves supplying these regions are directly related to

to the vessels. According to our observations, constriction of the vessels corresponded to strengthening of the impulsation and dilatation of the vessels to its inhibition.

DISCUSSION OF RESULTS

As seen from the facts which have been given, changes in the impulsation in the efferent nerves appear in a definite sequence. In order to understand the phenomena described, let us take as an initial unit the intensity of stimulation of the mechanoreceptors of the urinary bladder at which changes in the arterial pressure first appear. If this system is used, the changes in the impulsation in the various efferent nerves may be compared in a composite diagram as illustrated in Fig. 3, in which are included the results of all the experiments.

Figure 3 illustrates clearly the gradual implication of the individual vascular regions in the reaction as the intensity of stimulation of the mechanoreceptors of the urinary bladder is increased.

Examination of Fig. 3 shows that 0.25 conventional units is sufficient to implicate in the reaction the vessels of the urinary bladder itself. When the intensity of stimulation reaches 0.5 conventional units, the vessels of the kidney are implicated in the reaction. The vessels of both the urinary bladder and the kidney respond characteristically at this time by dilatation.

Further increase in the intensity of stimulation leads to strengthening of the impulsation to the vessels of the bladder and kidneys, i.e. vasoconstriction develops.

Increase of impulsation in the hypogastric nerve begins slightly earlier (0.7 conventional units) than in the renal nerves. It begins in the latter at a time when the first signs are observed of the reaction of the arterial pressure and requires 1.2 conventional units. At this time, as seen from the figure, the skin vessels and the vessels innervated by the cervical sympathetic nerve are included in the reaction. Obvious depression of impulsation is observed in these nerves.

Further study of Fig. 3 shows that as the intensity of stimulation increases and the magnitude of the reflexes affecting the arterial pressure becomes greater, the impulsation in the cutaneous and sympathetic nerves is still further depressed, the impulsation in the renal vessels continues to increase, whereas in the hypogastric nerves inhibition of impulsation reappears and is deeper than was observed at first.

The facts presented may thus be considered to show convincingly a movement of the process of excitation, to involve in the first place the centers most closely connected functionally with the particular interoceptive zone. In this way the vascular changes are confined to a relatively small area and changes in the general arterial pressure do not take place. Meanwhile, as the intensity of stimulation of the mechanoreceptors continues to rise, more and more new structures in the central nervous system become involved in the reaction in a definite order. This can be seen from the fact that soon after the vessels of the bladder are included in the reaction the renal vessels follow suit. If, however, the mechanism of the observed effects consisted purely of the gradual involvement of more and more new structures, it would be expected that an extraordinarily widespread involvement of the whole blood stream might take place which would not be without danger to the animal.

Our results show that side by side with the successive involvement of more and more new centers there are mechanisms which limit this irradiation.

Even at the very beginning, when the vessels of only the urinary bladder and kidneys were involved in the reaction, we can discern these mechanisms in the fact that initially the impulsation in the hypogastric and renal nerves is not strengthened but is inhibited (see Fig. 3). In other words, even in the beginning inhibition is created which limits the area of vasodilatation and therby prevents an increase in the arterial pressure.

When the rising intensity of stimulation causes this block to give way, the depression of the efferent impulsation is replaced by strengthening, but at this time inhibition is arising in other regions of the central nervous system, as shown by depression of the impulsation to the vessels of the skin and of the region innervated by the cervical sympathetic nerve (see the inhibition of the impulsation in these nerves shown in Fig. 3). With an even greater rise in the intensity of stimulation, inhibition of the efferent impulsation to the vessels of the urinary bladder arises once more and in consequence they are dilated. It is possible that this dilatation plays the part of a specific "protective" mechanism in preventing spasm of the vessels which may lead to prolonged ischemia of the organ.

Constant impulsation from the mechanoreceptors of the urinary bladder thus not only creates conditions for the irradiation of excitation but also promotes the onset of inhibition. The interaction of these two processes ultimately determines the reaction of the arterial pressure.

SUMMARY

Acute experiments were performed on cats. The changes of the efferent impulsations were studied in the sympathetic fibers innervating various vascular areas in vesicular mechanoceptor stimulation of different intensity.

Low intensity stimulation is required to produce changes in the impulsation of the nerves innervating the blood vessels of the urinary bladder proper (hypogastric nerve). With the rise of the intensity of stimulation these changes begin to spread to the more remote (in functional condition) vascular areas. At first the change of the efferent impulsation occurs to the renal vessels, then to the blood vessels of the skin of posterior extremity and, finally, to the vessels innervated by the cervical sympathetic nerve. With the rise of intensity of stimulation of the vesicular mechanoceptors these changes in the efferent impulsations in the hypogastric nerve undergo 3 phases: the first phase corresponds to the inhibition of the efferent impulsation, the second to its intensification and the third — to inhibition. Only the first two phases are observed in the renal nerve and only one in the skin and cervical sympathetic nerves. The intensification of the impulsation always leads to constriction while its inhibition — to the dilatation of the vessels.

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THE EFFECT OF AMINO ACIDS ON UNCONDITIONED INTEROCEPTIVE REFLEXES

COMMUNICATION I. THE RELATIONSHIP OF CENTER TO PERIPHERY IN THE MECHANISM OF INTEROCEPTIVE REFLEXES

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(Received March 28, 1958. Presented by Active Member of the AMN SSSR V. N. Chernigovskii)

The final form of a reflex is known to result from the interaction of three processes: the conditions of excitation of the receptors, the functional state of the central divisions of the reflex arc and the effective functional state of the organs responsible for performing the operation.

A considerable amount of material has now been collected which concerns this aspect of reflexes elicited by stimulation of the internal organs. Work by É. Sh. Airapet'yanets [1], I. A. Bulygin [3], M. L. Belen'kii [2] and V. N. Chernigovskii [12] has shown the importance of processes taking place in the receptor zone of the reflex and in the central nervous system.

N. A. Lapshin [7], investigating in 1948 reflexes caused by stimulation of the mechanoreceptors of the duodenum and rectum, found that they may appear in two forms: a fall or rise in the blood pressure. From analysis of this feature he established that this depended on whether the experiment was carried out on a starving or fed animal. After investigating the causes of this phenomenon, N. A. Lapshin [8] came to the conclusion that the changes in the reflexes could be explained by the difference in the pattern of impulses coming from the receptors in the alimentary tract of a fed or starving animal and also by the effect of certain chemical substances, circulating in the blood, on the functional state of the centers.

It was shown in work by V. V. Portugalov [10] that there are obvious differences in the state of the receptors Vater-Pacini corpuscles) of starving and fed animals, which can be demonstrated by histochemical methods. Recently work has been published by V. E. Delov, N. A. Adamovich, P. A. Kiselev and O. N. Zamyatina [4] which showed that the intensity of afferent impulses recorded in the celiac nerve of a cat depends on the functional state of the digestive system of the animal (the presence or absence of food in the alimentary tract, distension of the walls of the stomach with a rubber balloon). Meanwhile P. A. Kiselev, N. A. Adamovich and O. N. Zamyatina [6] have shown that the flow of afferent impulses in the sympathetic trunk of a frog depends on the functional state of the digestive system. A special investigation by O. N. Zamyatina [5] showed that in fed and starving animals there is a considerable difference in the pattern of impulses from the receptors. O. N. Zamyatina also established that the introduction of amino acids (leucine, cystine, tyrosine and tryptophane) into the lumen of the intestine in a concentration of 10^{-3} to 10^{-2} M causes the appearance of or the strengthening of existing afferent impulsation in the intestinal nerves. The receptors of the small intestine and pancreas are particularly sensitive to excitation by these substances.

V. A. Lebedeva [9] had previously shown that one of the amino acids — cystein — plays an important role in the mechanism of stimulation of receptors by chemical agents.

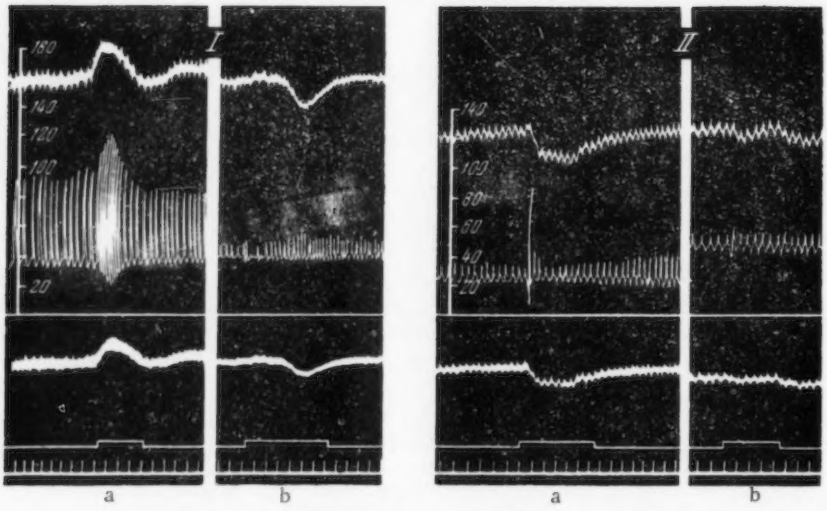

Fig. 1. Reflex changes in response to an increase of pressure in the duodenum. I) Transformation of the original pressor reaction (a) into depressor (b) after intravenous injection of glutaminic acid to a starving animal; II) transformation of the original depressor reaction (a) into pressor (b) after intravenous injection of glutaminic acid to a fed animal.
Significance of the curves (from above down): arterial pressure, respiration, zero line, pulse rate, stimulus marker (inflation of the duodenum), time marker (5 seconds).

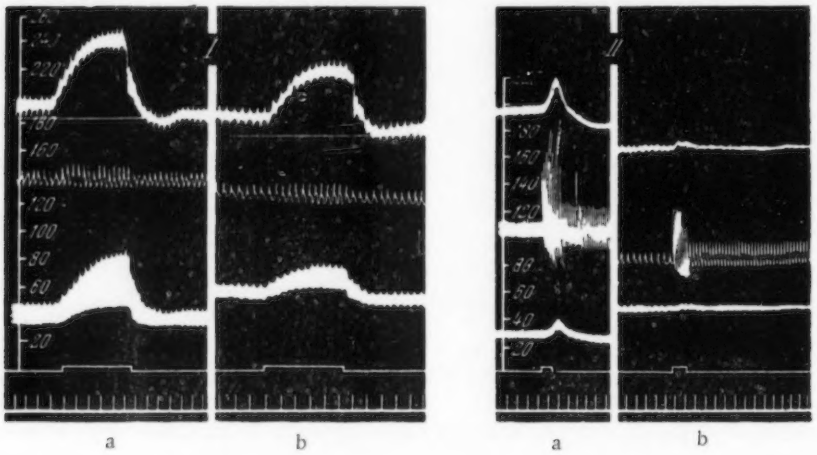

Fig. 2. Reflex changes during compression of the carotid artery.
I) Stimulation of the central end of the femoral nerve by an electric current; II) a) initial reactions; b) after intravenous injection of glutaminic acid.
Significance of the curves (from above down): arterial pressure, zero line of the reflex, respiration, pulse, stimulation marker which is also the zero line of the blood pressure, time marker (5 seconds).

In later work V. A. Tychinin [11] injected into the isolated carotid sinus of animals a mixture containing 10 amino acids (phenyl-α-alanine, glycocol, tryptophane, lysine, asparaginic acid, cysteine, glutaminic acid,

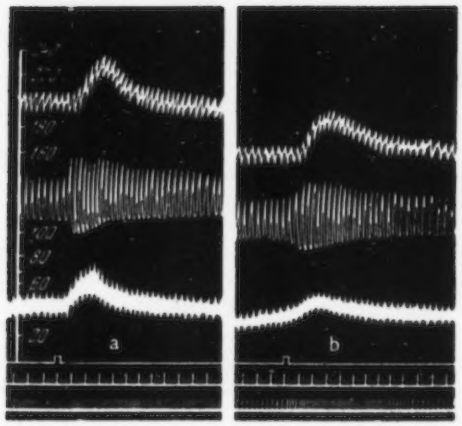

Fig. 3. Reflex changes during stimulation of the chemoreceptors of the small intestine. a) Initial reaction; b) after intravenous injection of glutaminic acid.
Significance of the curves (from above down): blood pressure, respiration, pulse, stimulation marker (administration of acetylcholine 10^{-5}), time marker (5 seconds) which is also the zero line, number of drops perfused.

tyrosine, histidine and norleucine) and observed a fall in the amino acid content of the blood which, in his opinion, was reflex in origin, as a result of stimulation of the carotid sinus receptors by the injected mixture.

Hence it might be thought that the action of amino acids could conveniently be used to investigate the relationship of center to periphery in the mechanism of interoceptive reflexes.

In the present work our object was to investigate whether changes in the reflexes similar to those observed by N. A. Lapshin [8] can be obtained by the direct action of amino acids on the centers or on the receptors.

EXPERIMENTAL METHOD

The investigation was carried out on fed and starving cats. The starving animals received no food for 2-3 days before the experiment. The fed animals were given a plentiful diet for 3-4 days before the experiment. Intravenous urethane anesthesia was used (1.5 - 2 ml of a 25% solution per 1 kg body weight).

Indicators of the magnitude of the reflex reactions were : the blood pressure, respiration, the rate of the heart. The blood pressure was recorded by a mercury manometer connected to the carotid artery of the animal. The rate of the heart was indicated by a membrane manometer. The respiration was recorded by means of a Marey's capsule connected to the animal's trachea. The reflexes caused by stimulation of the mechano and chemoreceptors of the intestine, the reaction to clamping the carotid artery and the reflex caused by stimulation of the central end of the femoral nerve with an electric current were studied. Stimulation of the mechanoreceptors of the duodenum, with its nervous and humoral connections with the body intact,was carried out by inflating it with air to a pressure of 60 -80 mm of mercury.

The chemoreceptors of the perfused segment of the small intestine were stimulated by application of acetylcholine or nicotine in doses of 5 - 50 γ.

Compression of the carotid artery opposite to that in which the blood pressure was recorded was carried out by means of a special clamp for a period of 20 - 25 seconds. The central end of the femoral nerve was stimulated by an electric current from the secondary winding of an induction coil (4v, 50 impulses per sec, distance between coils 15 - 17 cm).

Solutions of neutralized L-stereoisomers (glutaminic acid, cysteine, alanine) in a concentration of 1: 10^{-3} were injected singly into the femoral vein. Altogether 87 experiments were performed.

EXPERIMENTAL RESULTS

The research of O. N. Zamyatina showed that introduction of amino acids into the lumen of the intestine causes a marked strengthening of impulsation. It might be considered that in N. A. Lapshin's experiments the changes in the reflexes were associated with a change in the functional state of the centers or the periphery following the appearance of amino acids in the blood of the fed animals.

It was therefore important in the first place to test the effect of intravenous injection of amino acids on the unconditioned interoceptive reflexes. It had to be ascertained whether amino acids had any effect on the blood pressure and respiration by direct action on the centers or periphery or both.

In the first series of experiments we studied the reflexes caused by stimulation of the mechanoreceptors of the duodenum in fed and starving animals after the intravenous injection of amino acids. The experiments

showed that the intravenous injection of amino acids to a starving animal (glutaminic acid, cysteine or alanine) leads to a replacement of the pressor reflex, previously observed in response to stimulation of the duodenum by a depressor reflex (Fig. 1, I, a, b). Injection of amino acids to a fed animal elicited a depressor reflex instead of a pressor (Fig. 1, II, a, b). In these experiments the amino acids could exert their effect either centrally or peripherally on the receptors of the duodenum which retained its nervous and humoral connections with the body.

In the second series of experiments we set out to study the possible action of injection of amino acids on the centers. We investigated the reflex to compression of the carotid artery. Fig. 2, I, a, b shows that in some cases the initial pressor reflex to compression of the carotid artery either diminishes slightly or increases and in other cases the pressor reactions are transformed into depressor. Thus in this series of experiments no conclusive results were obtained. It follows from these experiments that the central action of amino acids is not of decisive importance in influencing the direction of the reflexes.

In the third series of experiments (Fig. 2, II, a, b) the central end of the femoral nerve was stimulated with its contained somatic afferent fibers. After injection of amino acids the reflex action on the blood pressure was considerably depressed in both fed and starving animals. The results of this series of experiments showed that the peripheral elements of the reflex arc were concerned in the changes in the interoceptive reflexes after injection of amino acids. In order to settle once and for all the question of the degree of participation of the centers and periphery in influencing the direction of the reflexes, it was decided to examine changes in the reflexes from the chemoreceptors of the perfused segment of small intestine, retaining only its nervous connections with the body after the intravenous injection of one of the amino acids which we were using. Fig. 3 shows the kymogram of one of these experiments.

In these experiments the amino acid was not in contact with the receptors and the reflex action on the blood pressure and respiration was practically unchanged. These experiments demonstrate that central mechanisms are not the only ones in carrying out the interoceptive reflexes.

These results show that the injection of amino acids in very small amounts is capable of substantially influencing the interoceptive reflexes. As the investigations showed, the intravenous injection of amino acids alters the character of the reflexes, mainly by affecting the peripheral part of the reflex arc. This is shown by the experiments in which the reflexes from the duodenum and femoral nerve were altered after the intravenous injection of amino acids. The experimental results showing absence of changes during stimulation of the chemoreceptors of the perfused segment of the intestine after intravenous injection of amino acids do not support the view that the centers play a major role.

Decisive components of the mechanism of the interoceptive reflexes are evidently a change in the metabolic state of the receptive zone and the impulsation passing from that zone to the central nervous system.

SUMMARY

The aim of this work was the study of the effect of amino acids on the unconditioned interoceptive reflexes. The work was conducted on fasting cats and on those which were fed in condition of urethane anesthesia. Neutralized solutions of glutamic acid, cysteine and alanine were injected intravenously in the $1 : 10^{-3}$ concentration. The author studied the changes of the reflex reactions in response to the stimulation of duodenal mechanoreceptors, the chemoreceptors of the perfused section of the small intestine, the baroreceptors of carotid sinus and in response to the electric stimulation of the central end of the femoral nerve. After the intravenous administration of amino acids to fasting animals a change of the blood pressure pressor reactions into depressor was noted, while in animals which were fed the depressor reactions changed into pressor. The pressor reflex to stimulation of the femoral nerve was depressed both in fasting cats and in those which were fed. In stimulation of the baroreceptors of carotid sinus in these conditions the reflexes did not follow any definite laws.

Reactions in response to the stimulation of the chemoreceptors of the perfused portion of intestine did not change. Thus, the intravenous administration of amino acids has a definite effect on the character of the reflexes, mainly by changing the condition of the peripheral part of the reflex arc,.

Evidently, the change in the metabolic processes occurring in the receptive zones and the impulsation which is transmitted from the zone to the central nervous system are decisive in the mechanism of interoceptive reflexes.

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PATHOLOGICAL PHYSIOLOGY AND GENERAL PATHOLOGY

THE MECHANISM OF EXCRETION OF DYSENTERY ANTIGEN BY THE KIDNEYS

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(Received May 17, 1958. Presented by Active Member of the AMN SSSR L. A. Zil'ber)

Many investigations have been devoted to the excretion of viruses and bacterial antigens by the kidneys. These have supplied information about viruses and antigens excreted, but not about the mechanisms concerned in their excretion. Only in recent years has research been undertaken, under the direction of L. A. Zil'ber and A. D. Ado, into the mechanisms of excretion of viruses and antigens by the kidneys [1-5].

The objects of our present investigation were: to study the influence of proteinuria due to hyperproteinemia on the process of excretion of antigen of the Flexner dysentery bacillus; to ascertain the laws governing the excretion of antigen during maximum secretion of diodrast; and to discover any changes in the excretion of antigen during selective poisoning of the cells of the tubules by 2,4-dinitrophenol.

EXPERIMENTAL METHOD

Hyperproteinemia was induced by the repeated intraperitoneal injection of homologous serum in doses of 50, 70, 90 and 100 ml per 1 kg body weight of the animal, with intervals of 2-3 days between injections. The plasma proteins were determined with a refractometer. The quantity of protein in the urine was studied by the Roberts-Stol'nikov method. The concentration of diodrast in the blood was kept between 15-30 mg% by infusion of a solution or by repeated injection of a 10% solution in small doses (6-10 ml) in each clearance period of 20 minutes. Diodrast was determined by the method described by N. A. Ratner [6]. The maximum secretion of diodrast was calculated from the formula

$$K_{MS} = K_M D - 0.73\, K_P F_K,$$

where K_{MS} – maximum secretion; K_M – concentration of diodrast in the urine; D – the minute diuresis; K_P – concentration of diodrast in the plasma; F_K – filtration as determined by creatinine; 0.73 – readily soluble fraction of diodrast excreted by filtration (coefficient of filtration of diodrast).

Poisoning with 2,4-dinitrophenol was carried out with 10 mg of the drug per 1 kg body weight of the animal. In the experiments we used healthy dogs whose kidneys had been shown to be free from any abnormality of function by repeated investigations.

Complete Flexner dysentery antigen, obtained from the I. I. Mechnikov Moscow Institute of Vaccines and Sera, was injected intravenously or intramuscularly in a dose of 0.1 - 0.4 mg per 1 kg body weight of the animal. The quantity of antigen in the serum and urine was estimated by the cold complement fixation reaction. Precipitated polyvalent dysentery serum, with a titer according to the complement fixation reaction of 1: 2,000,000, obtained from the Moscow Institute of Vaccines and Sera, was used in the performance of the reactions. By the use of this serum it was possible to detect the presence of 0.0000005 g or 0.0005 mg of antigen in 1 ml of fluid tested.

TABLE 1

The Effect of Proteinuria on the Excretion of Dysentery Antigen by the Kidneys

Name of dog	Minute diuresis ml/min	Creatinine clearence index ml/min	Antigen				Plasma proteins				
			concentration in serum, mg%	concentration in urine, mg%	concentration index	clearance index, ml/min	concentration in blood	concentration in urine, %	filtration, mg/min	excretion in urine mg/min	reabsorption mg/min
Roza	0.8	68	0.4	0.2	0.5	0.4	—	—	—	—	—
Knopka	0,5	66.4	0.4	0.2	0.5	0.25	—	—	—	—	—
Volna	0.7	91.8	0.2	0.1	0.5	0.35	—	—	—	—	—
Norma	0.5	98.7	0.4	0.2	0.5	0.25	—	—	—	—	—
Roza	0.7	62	0.4	0.8	2	1.4	10.28	0.65	27.34	4.55	23.79
Knopka	0,4	66	0.4	0.8	2	0.8	9.78	0.33	27.1	1.32	25.8
Volna	0,41	71.7	0.2	0.4	2	0.82	10.80	0.264	33.18	1.08	32.1
Norma	0.5	80.5	0.4	0.4	1	0.5	9.81	0.429	33.8	2.14	31.7
Lapusha	1,0	96	0.4	0.8	2	2.0	—	—	—	—	—
Laika	1.0	108	0.4	0.8	2	2.0	—	—	—	—	—
Laska	0.6	74	0.4	0.8	2	1.2	—	—	—	—	—
Roza	0.5	68	0.4	0.8	2	1.0	—	—	—	—	—
Lapusha	1.0	98	0.4	3.2	8	8.0	10.41	0.165	43.85	1.65	42.2
Laika	0,7	105	0.8	6.4	8	5.6	10.69	0.099	48.12	0,92	47.2
Laska	0,4	66	0.4	3.2	8	3,2	10.85	0,099	28.7	0.39	28,3
Roza	0.5	68	0.4	3.2	8	4.0	10.17	0.132	29.64	0.66	28.9

TABLE 2

The Excretion of Dysentery Antigen by the Kidneys of Dogs during Maximum Secretion of Diodrast

Name of Dog	Minute diuresis ml/min	Creatinine clearance index ml/min	Antigen				Diodrast				
			concentration in serum, mg%	concentration in urine mg%	concentration index	clearance index ml/min	concentration in plasma mg%	concentration in urine, mg%	filtration, mg/min	maximum secretion, mg/min	excretion in urine mg/min
Laika	2.0	113.0	0.4	0.8	2	4	—	—	—	—	—
Malyshka	1.9	91.0	0.4	0.8	2	3.8	—	—	—	—	—
Lapusha	1.5	92	0.4	0.8	2	3.0	—	—	—	—	—
Laika	2.1	113.6	0.4	1.6	4	8.4	19.7	4 000	16.33	67.6	84.0
Malyshka	2.0	100	0.4	1.6	4	8.0	16.1	4 770	11.75	83.6	95.4
Lapusha	1.0	102.3	0.4	1.6	4	4.0	30.5	7 890	22.7	56.1	78.9

EXPERIMENTAL RESULTS

In the first series of investigations the influence of proteinuria on the excretion of dysentery antigen was studied in 8 dogs.

As seen from Table 1, proteinuria increases the excretion of dysentery antigen by the kidneys of both immunized and unimmunized dogs, but this effect is much more marked in the immunized animals.

In unimmunized dogs the antigen is excreted slowly the concentration index is 0.5, the clearance index varies between 0.25 and 0.4 ml/min. In proteinuria due to hyperproteinemia the antigen is excreted more intensively; the concentrating power of the kidneys in respect of the antigen is increased to give an index of 2; with diuresis close to that of the control experiment, clearance increases and varies between 0.5 and 1.4 ml/min. After active immunization the influence of proteinuria on clearance of antigen is still more pronounced. Under identical experimental conditions, with equal diuresis and the same concentration of antigen in the plasma, proteinuria brings about a marked increase in the antigen clearance index, which reaches 4-8 ml/min compared with 0.5-1.4 ml/min in unimmunized animals.

The increased excretion of dysentery antigen during proteinuria, due to hyperproteinemia, is evidently explained by the greater reabsorption of serum protein than of antigen. An explanation of the mechanism of this relationship between reabsorption of antigen and of serum protein requires a special investigation.

Some workers [7] have shown that secretion of para-aminohippuric acid by the tubule cells leads to depression of reabsorption of ascorbic acid. In view of this finding, in the next series we attempted to explain the effect of maximum secretion of diodrast on the process of excretion of dysentery antigen in the final urine. The results of experiments performed on 3 dogs are shown in Table 2. As seen from this table, maximum secretion of diodrast is accompanied by slight stimulation of the excretion of dysentery antigen by the kidneys of dogs. In experiments without diodrast and with the same concentration of antigen in the serum in immunized animals, the concentration index was 2 and the clearance 4 ml/min. With maximum secretion of diodrast and the same concentration of antigen and minute clearance, the concentration index rose to 4 and the clearance to 8.4 ml/min.

The results of this series of experiments show that maximum secretion of diodrast is accompanied by a considerable increase in the excretion of dysentery antigen by the kidneys of immunized dogs. How can this

TABLE 3

The Excretion of Dysentery Antigen by the Kidneys of Dogs during Poisoning with 2,4-Dinitrophenol

Name of dog	Dose of 2,4-dinitrophenol, mg/kg	minute diuresis, ml/min	Creatinine clearance index, ml/min	Antigen			
				concentration in serum, mg%	concentration in urine, mg%	concentration index	clearance index ml/min
Lapusha	—	1.8	94	0.4	0.8	2	3.6
Mal'va	—	2.2	110	0.4	0.8	2	4.4
Malyshka	—	1.5	114	0.4	0.8	2	3.0
Laika	—	1.6	102	0.4	0.8	2	3.2
Lapusha	10	1.9	96	0.4	1.6	4	7.6
Mal'va	10	2.0	110	0.8	3.2	4	8.0
Malyshka	10	1.5	112	0.4	1.6	4	6.0
Laika	10	1.5	—	0.4	1.6	4	6.0

mechanism of increased antigen clearance during maximum secretion of diodrast be explained? According to the same workers [7] maximum secretion of one substance inhibits the reabsorption of another.

If this view[7] is accepted, it can be assumed that in our experiments the increased excretion of antigen is connected with some degree of inhibition of its reabsorption from the lumen of the tubules.

On these grounds it may be postulated that some particles of dysentery antigen pass through the glomerular membrane and part of these are reabsorbed during their passage along the tubules. Reabsorption of antigen by the cells of the tubules may be inhibited by maximum secretion of diodrast or stimulated by reabsorption of protein; as a result of this much more antigen is excreted in the final urine.

In our next series of investigations we studied the effect of selective poisoning of the tubule cells during excretion of dysentery antigen by the kidneys of dogs. The animals were poisoned with 2,4-dinitrophenol, which in a dose of 10 mg per 1 kg body weight causes selective damage to the cells of the tubules. As we showed, in spite of the early development of a febrile reaction to the 2,4-dinitrophenol, signs of tubular damage were seen from 2-3 hours after the beginning of its administration. Accordingly we gave dinitrophenol to the animals from 2-3 hours before injecting the antigen. Experiments on 4 immunized dogs enabled us to discover certain distinctive features of the excretion of antigen during poisoning of the tubule cells. In Table 3 are shown the experimental results obtained after poisoning the dogs with 2,4-dinitrophenol.

In immunized animals poisoned with 2,4-dinitrophenol the concentration index was twice as high as in the previous experiments (before poisoning).

The blood antigen clearance value is also correspondingly raised to 6-8 ml/min. The increased excretion of dysentery antigen in the poisoned animals is evidently connected with a fall in reabsorption of antigen, since in the doses in which we administered it, 2,4-dinitrophenol causes selective damage to the cells of the tubules without affecting the permeability of the glomerular membrane.

From an analysis of all the results it may be concluded that many particles of dysentery antigen pass through the glomerular membrane without damaging it. During its passage along the tubules part of the antigen is resorbed by the cells of the proximal division of the tubules by the same mechanism as that responsible for the reabsorption of protein present in the lumen of the tubules. The intensity of reabsorption of antigen depends on the immunological state of the animal and also on the processes of secretion and reabsorption of other substances (components) passing through the kidney at the same time as the antigen.

SUMMARY

Proteinurea caused by hyperproteinemia considerably intensifies the excretion of the dysentery antigen by the dog's kidneys. The mechanism of increased urinary excretion of the antigen in hyperproteinemia is connected

with its reduced resorbtion. The maximal secretion of cardiotrast as a result of development of competitive relations with the antigen reabsorption unhibitis the absorption of the latter and increases its excretion into the final portion of the urine. 2,4-Dinitrophenol poisoning causes a selective affection of the tubular cells and limits the resorbtion of the antigen.

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THE ACCUMULATION OF SULFATHIAZOLE IN INFLAMMATORY FOCI OF THE SKIN IN ANIMALS WHILE ASLEEP AND AWAKE

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In recent years drug-induced sleep has been widely used in the treatment of many diseases.

It is known that inhibition of the cerebral cortex of long or short duration may have a favorable effect on the development and course of certain pathological reactions [4-6, 9 and others]. It has also been shown that during sleep changes occur in the course of many physiological processes in man and animals. N. I. Leporskii observed a marked fall (tenfold) in the gastric secretion in healthy persons during sleep [8]. In rabbits, during sleep induced by medinal, the pulse is slowed, the respirations are less frequent and the body temperature falls [10]. It has further been shown that in rabbits during drug-induced sleep the concentration of penicillin in the blood and organs is higher than when awake, and the excretion of the drug in the urine of animals takes place more slowly [2, 3, 10, 13].

Investigations of the blood and urine of dermatological patients treated with sulfonamides have shown that at night, during sleep, the concentration of sulfonamides in the blood is considerably higher than during the daytime and the excretion of the drug in the urine of patients takes place more slowly during sleep. V. A. Kozlov observed delay in the excretion of ink particles from the peritoneal cavity of anesthetized animals [7]. S. Sh. Sakanyan observed increased absorptive powers of the reticuloendothelial system towards congo red in the majority of a series of animals under morphine-chloroform-ether anesthesia. He also showed that painful stimulation lowers the absorptive powers of the reticuloendothelial cells [14, 15]. Painful stimulation is known to be accompanied by severe constrictions of capillaries [1], which may be reflected in the deposition of dyes and drugs in the tissues.

In the present work we studied the influence of drug-induced sleep on the accumulation of sulfathiazole in foci of inflammation in the skin. For this purpose we carried out 3 series of experiments on 26 adult rabbits.

EXPERIMENTAL METHOD AND RESULTS

First series of experiments: accumulation of sulfathiazole in foci of inflammation in the skin due to the action of a 10% mixture of chloroform in alcohol in rabbits while asleep and awake. Application of a 10% mixture of chloroform in alcohol (9 parts chloroform by volume and 1 part of 96° ethyl alcohol) causes a sensation of burning and pain and later forms an inflammatory lesion of the skin, terminating by necrosis of the superficial or deep parts of the skin, depending on the exposure [11, 12]. Knowing these properties, we employed this mixture and investigated its effect on the development of inflammation and on the deposition of a drug in the skin of animals during sleep. Observations were made on 9 rabbits: 6 rabbits were given intravenous injections of hexobarbitone (5% solution) in a dose of 100 mg per 1 kg body weight and 3 rabbits were controls, i.e. did not receive the drug.

Sleep developed in the animals a moment after injection of the drug. As soon as they were asleep, these animals together with the controls were injected intravenously with 4 ml each of a 3% solution of sodium

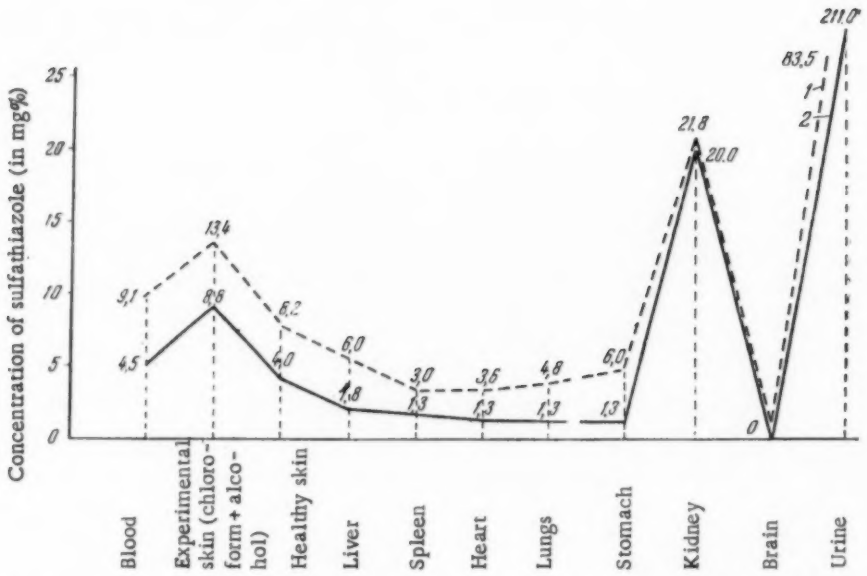

Fig. 1. Content of sulfathiazole (in mg%) in the blood, organs and urine of rabbits while asleep and awake, 1 hour after injection of the drug and application of a 10% mixture of chloroform in alcohol to the skin.
1) Sleeping animals; 2) waking animals.

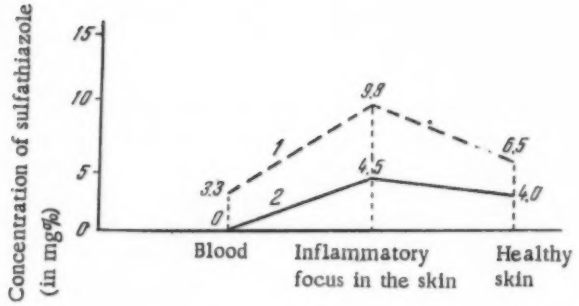

Fig. 2. Mean concentration of sulfathiazole (in mg%) in the blood, the inflammatory focus in the skin and the healthy skin of rabbits while asleep and awake.
1) Sleeping animals; 2) waking animals.

sulfathiazole per 1 kg body weight, and at the same time to the shaven skin of the lateral surface of the trunk was applied for 2 minutes a gauze swab soaked in a 10% mixture of chloroform in alcohol. All the control rabbits and some of the rabbits in a state of sleep reacted to the chloroform-alcohol mixture (they shuddered and twiched at the side).

At the site of application of the mixture the control rabbits developed clear erythema and edema of the skin; in the sleeping rabbits the erythema was less clear and the edema less marked. One hour after injection of sulfathiazole, 2 ml of blood was taken from the auricular vein and the animals were killed by injection of air into the vein. The organs of the animals were examined for their sulfathiazole content by A. M. Timofeeva's method.

During the investigation it was found that the sulfathiazole concentration in the skin at the site of action of the chloroform-alcohol mixture (in the experimental rabbit) and also in all the organs and blood of the animals in a state of hexobarbitone-induced sleep, was 2 or 3 times higher than that in the control animals (Fig. 1). The concentration of the drug in the urine of the animals during sleep was $2\frac{1}{2}$ times lower than when awake.

The percentage of combined sulfathiazole in the urine of the sleeping animals was just over half (24%) that in the controls (45%). The increased concentration of the drug in the tissues of the sleeping animals, and the lower percentage of combined sulfathiazole indicate that in these animals during sleep the processes of combination of the drug are depressed and excretion of the drug from the body is slowed.

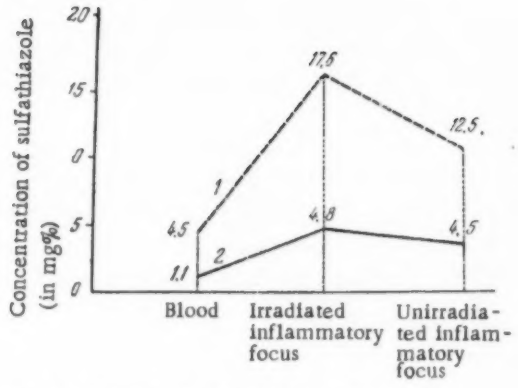

Fig. 3. Content of sulfathiazole in the inflammatory foci of rabbits irradiated by a Sollux lamp while asleep and awake. 1) Animals irradiated while asleep; 2) animals irradiated while awake.

Second series of experiments: accumulation of sulfathiazole in foci of suppurative inflammation of the skin in rabbits while asleep and awake. There were 10 rabbits in this experiment. All the animals were inoculated subcutaneously with 10^8 organisms from a 48-hour agar culture of a hemolytic Staphylococcus aureus. After 24 hours well marked foci of inflammation were seen at the site of injection of the culture of microorganisms, with the appearance of localized erythema and infiltration of the skin and a central pustule. The diameter of the foci of inflammation did not exceed 2-3 cm.

Six rabbits received intravenous injections of 5% hexobarbitone solution in a dose of 100 mg per 1 kg body weight. At the same time they were injected intravenously with a dose of 4 ml per 1 kg body weight of a 3% solution of sodium sulfathiazole. The remaining four (control) rabbits received sulfathiazole alone, without hexobarbitone. One hour after the injection of sulfathiazole an operation was performed on all the rabbits (the focus of inflammation and a piece of healthy skin were excised from the animals). Blood was taken from the auricular vein 2 hours after the injection of sulfathiazole. During the investigation we found the following concentrations of sulfathiazole (Fig. 2): in the control animals in the inflammatory focus — 4.5 mg%, in healthy skin — 4 mg% and in the blood —0. In the animals receiving hexobarbitone (i.e. sleeping), the concentrations of the drug were significantly higher than in the controls: in the inflammatory focus — 9.8 mg%, in healthy skin — 6.5 mg% and in the blood — 3.3 mg%. The higher concentration of the drug in the tissues of the sleeping animals indicated that accumulation of sulfathiazole in the tissues is more strongly marked in animals during sleep.

Third series of experiments: accumulation of sulfathiazole in foci of inflammation of the skin in animals under the influence of irradiation by a Sollux lamp while asleep and awake. There were 7 rabbits in the experiment, and in each of them 2 suppurative inflammatory foci were induced in the skin by intradermal injection of 10^8 microorganisms from a 24-hour agar culture of a hemolytic Staphylococcus aureus into the lateral surface of the trunk. 24 hours after inoculation, 4 rabbits were given intravenous injections of hexobarbitone (5% solution) in a dose of 100 mg per 1 kg body weight; the 3 remaining rabbits did not receive hexobarbitone and acted as controls. Immediately after falling asleep all the rabbits (sleeping and controls) were injected intravenously with a dose of 4 ml per 1 kg body weight of a 3% solution of sodium sulfathiazole, and at the same time one inflammatory focus was irradiated for 15 minutes by a Sollux lamp at a distance of 20 cm. As a result of irradiation the erythema of the skin at the foci of inflammation was intensified. All the control rabbits reacted with pain to the irradiation (they twitched at the side, trembled and tried to get away from the apparatus). Two hours after the injection of sulfathiazole, 2 ml of blood was taken from the auricular vein of the animals and the foci of inflammation in the skin were excised.

The following concentrations of sulfathiazole were established by the investigation (Fig. 3): in control animals — in the blood — 1.1 mg%, in the irradiated inflammatory focus — 4.8 mg%, unirradiated — 4.5 mg%; in the experimental rabbits (receiving hexobarbitone) — in the blood — 4.5 mg%, in the irradiated inflammatory focus — 17.6 mg%, unirradiated — 12.5 mg%, i.e. in the last series of animals the concentration of sulfathiazole in the tissues examined was almost 4 times as high as in the first series. The amount of free sulfathiazole in the sleeping animals reached 55 mg% in the blood, 74 mg% in the irradiated inflammatory focus and 88 mg% in the unirradiated focus; meanwhile the values for the control animals were: in the blood — 0, in the irradiated inflammatory focus — 48 mg% and in the unirradiated focus — 62 mg%. The higher level of free sulfathiazole in the blood of the sleeping rabbits indicates a slowing of the processes of combination of sulfathiazole in these animals.

From the experiments described the following conclusions may be drawn.

In animals receiving painful stimulation by a mixture of chloroform and alcohol while asleep, the concentration of sulfathiazole in the tissues is higher than in animals similarly stimulated while awake, and under these circumstances the sulfathiazole concentration in the inflammatory foci in the skin is higher in the sleeping than the waking animals.

The sulfathiazole concentration in the inflammatory foci in the skin is higher in animals irradiated with a Sollux lamp while asleep than in those irradiated while awake, and the sulfathiazole content of the urine in sleeping animals is $2\frac{1}{2}$ times lower than in waking animals, which may indicate a depression of the excretory function in animals in a state of sleep.

The lower percentage of combined sulfathiazole in the tissues of sleeping animals is evidence of retardation of the combination of sulfathiazole in these animals.

SUMMARY

The accumulation of sulphathiazol was studied in inflammatory foci of the skin of rabbits during sleep. The injury was inflicted with 10% mixture of chloroform and alcohol applied to the skin or by the intradermal injection of staphylococcus culture. Sleep was induced by the administration of hexabarbitone. The animals were sacrificed or operated in 1-2 hours after the administration of sulphathiazol. The concentration of sulphathiazol in the blood, inflammatory foci and internal organs was found to be greater during sleep than while being awake. The irradiation of the inflammatory foci by ultraviolet rays during sleep resulted in intentified accumulation of sulphathiazol in the inflammatory foci as compared to the accumulation in the animals irradiated while awake.

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BIOCHEMISTRY AND BIOPHYSICS

THE INFLUENCE OF THE ADRENOCORTICOTROPIC HORMONE OF THE PITUITARY GLAND ON CERTAIN ASPECTS OF LIPID, CARBOHYDRATE AND NITROGEN METABOLISM IN DOGS

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The adrenocorticotropic hormone of the pituitary gland(ACTH) is known to activate mainly the formation of glucocorticoids (corticosterone and hydrocortisone) in the adrenal cortex. Reports in the literature [1, 2, 6] point out that the glucocorticoids of the adrenal cortex affect several aspects of lipid, carbohydrate and nitrogen metabolism, mainly influencing gluconeogenesis from protein and causing deposition of glycogen in the liver [3, 4, 5]. Information in the literature on the effect of ACTH on metabolic processes is contradictory, evidently because of failure to take into account the varying degree of activation of the adrenal cortex by this hormone and the possible development not only of stimulation but also of depression of the functional power of the adrenal cortex during the prolonged administration of ACTH.

In the present work we aimed to explain still further the influence of administration of ACTH of Soviet origin on certain aspects of lipid, carbohydrate and nitrogen metabolism.

EXPERIMENTAL METHOD

As experimental animals we used dogs weighing 5.5 - 10 kg. The ACTH preparation was injected subcutaneously in different doses. In the first series of experiments we investigated the effect of a single injection of ACTH on the content of sugar, cholesterol, ketone bodies, residual nitrogen and amino acid nitrogen in the blood 3 and 5 hours after injection of ACTH, and also on the daily urinary excretion of total nitrogen, amino acid nitrogen, creatine, creatinine and neutral 17-ketosteroids. In a second series of experiments we studied the effect of repeated (over a period of 5-10 days) injection of ACTH and in a third series — the influence of this drug on a background of increased metabolism caused by administration of phenamine and thyroidin. In all the experiments a series L-4 preparation was used with an activity of 1 unit/mg determined by the State control laboratory of our Institute. Furthermore, in each experiment the activity of the preparation was checked by the eosinopenic reaction which it caused.

EXPERIMENTAL RESULTS

I. Experiments (46 in number), carried out on 9 dogs, show that the changes in the content of sugar, ketones, cholesterol, amino acids and nitrogen in the blood in the 3-5 hours after a single subcutaneous injection of ACTH in doses of 5-15 units per 1 kg body weight are within the normal limits of variation. Under these circumstances, in the majority of the experiments the following regular feature is observed: when the initial level of the above constituents of the blood is at the lower limit of normal the injection of ACTH increases this level to the upper limit of normal (Fig. 1, a and b, curve 1; Fig. 2, curves 1 and 2). If the initial level of these constituents is at the upper limit of normal, injection of ACTH lowers it (see Fig. 1, a and b, curve 2; Fig. 2, curves 1, a and 2, a).

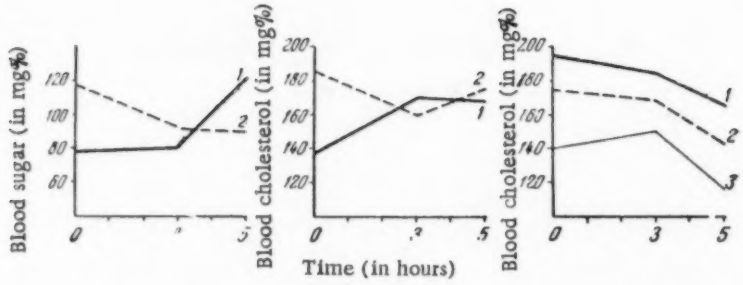

Fig. 1. Changes in the blood sugar (a) and cholesterol (b) in dogs 3 and 5 hours after injection of ACTH in a dose of 5 units per 1 kg body weight, when the initial level was high (1) and low (2); (c) blood cholesterol 3 and 5 hours after injection of ACTH in a dose of 15 units per 1 kg body weight, for various initial levels: (1) for an initial level of 194 mg%; (2) for an initial level of 175 mg%; (3) for an initial level of 140 mg%.

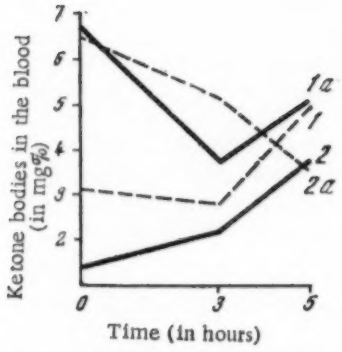

Fig. 2. Changes in the content of ketone bodies in the blood of dogs with a low (1) and high (1a) initial level, 3 and 5 hours after injection of ACTH in a dose of 5 units per 1 kg body weight; with a low (2) and high (2a) initial level, 3 and 5 hours after injection of ACTH in a dose of 15 units per 1 kg body weight.

The changes in the nitrogenous constituents of the urine after a single injection of ACTH stand out in greater relief. The nitrogen content of a 24-hour sample of urine increases as a rule after the injection of both 5 and 15 units per 1 kg body weight. If, however, in this particular dog the initial (before injection of ACTH) nitrogen excretion was above the average level, injection of ACTH in a series of experiments reduces this excretion (Table 1). The same feature is regularly observed with excretion of amino acids: when the amino acid content of the urine is low injection of ACTH usually increases the excretion of amino acids in the urine; if the amino acid nitrogen excretion is raised considerably, injection of the same dose of ACTH causes a fall in its content in a 24-hour sample of urine. However it must be emphasized that injection of relatively large doses of ACTH (15 units per 1 kg body weight) increases the excretion of amino acid nitrogen in those cases when the initial content of this in the urine is raised (see Table 1).

When relatively large doses of the preparation (15 units per 1 kg body weight) are used the changes in the blood cholesterol and the content of amino acids in the urine show a definite directional trend: the blood cholesterol falls slightly (see Fig. 1, c, curves 1, 2, 3), and the amino acid content of the urine rises (see Table 1, dogs Nos. 3, 6 and 10). The first must be considered to be connected with utilization of cholesterol in the formation of glucocorticoids in the adrenals, and the second is evidently due to increased decomposition or to interference with the resynthesis of protein.

II. Repeated (over a period of 10 days) injection of ACTH in a dose of 5 units per 1 kg body weight daily (experiments on 7 dogs) causes changes in the content of sugar, ketone bodies, residual nitrogen and amino acid in the blood which are within normal limits of variation. At the same time it must be emphasized that when the initial level of these constituents is relatively low, during the first stage (5 days) of its injection, ACTH causes a rise and later (until the 10th day) a fall once more.

When the initial blood level of these constituents is relatively high, during prolonged administration of ACTH their content becomes closer to the average normal value.

TABLE 1

The Effect of a Single Injection of ACTH on the Total Nitrogen and Amino Acid Nitrogen Excretion in the Urine

Dog No.	Dose of ACTH in units per 1 kg body weight	Daily excretion of nitrogen in the urine (in g)		Dog No.	Dose of ACTH in units per 1 kg body weight	Daily excretion of amino acid nitrogen in the urine (in mg)	
		before injection of ACTH	after injection of ACTH			before injection of ACTH	after injection of ACTH
1	5	3.8	7.5	3	5	90	139
3	5	3.7	7.4	5	5	58	134
5	5	4.7	7.0	2	5	52	137
8	5	6.2	11.5	8	5	425	209
8	5	6.5	9.1	7	5	118	102
6	15	4.5	7.1	3	15	150	320
6	15	8.2	5.7	3	—*	162	169
3	15	13.2	8.5	6	15	173	281
3	—*	11.1	11.4	10	15	163	270
				10	15	150	212

* Control (no ACTH injected). Examined at the same times as the experimental dogs.

Under these experimental conditions cholesterol in the majority of cases shows a tendency to fall (Table 2).

The changes in the content of nitrogenous constituents of the urine are characteristic. Whereas the repeated injection of ACTH over a period of the first 5 days causes a significant increase in the total nitrogen and the amino acid nitrogen excreted in the urine, further (for the next 5 days) injection of ACTH results in a fall in their excretion. In dog No. 2, for instance, before injection of ACTH the total nitrogen content was 3.2 g in 24 hours, on the 5th day after injection of ACTH it was 8.8 g in 24 hours and on the 10th day after injection of ACTH the total nitrogen content fell to 1.3 g in 24 hours. The corresponding amino acid nitrogen content was: before the experiment 53 mg in 24 hr, on the 5th day after injection of ACTH it was 76 mg in 24 hours and on the 10th day after injection of ACTH it had fallen to 37 mg in 24 hours. The same thing was observed in dog No. 1.

In the animals in which the initial total nitrogen and amino acid nitrogen contents in the urine were high, prolonged injection of ACTH causes a fall in their excretion. Before injection of ACTH, dog No. 4, for instance, excreted in the urine in 24 hours 13.2 g of total nitrogen, on the 5th day after injection of ACTH it excreted 7.8 g in 24 hours and on the 10th day — 2.7 g in 24 hours. The amino acid nitrogen content of another dog (No. 5) was: before the experiment — 87 mg in 24 hours, on the 5th day after injection of ACTH — 79 mg in 24 hours and on the 10th day — 52 mg in 24 hours.

Thus after both single and repeated injections of ACTH, in the majority of experiments this hormone has a normalizing effect on the various indicators of lipid, carbohydrate and nitrogen metabolism which we investigated.

III. It must be mentioned that the normalizing effect of ACTH on the various aspects of nitrogen metabolism is also shown in cases when this has been deranged by suitable experimental procedures. Two examples will be given. After administration of phenamine (1 mg per 1 kg body weight daily for 5 days) there is an increase in the total nitrogen, amino acid nitrogen, creatine and creatinine excreted in the urine, and a single injection of ACTH in a dose of 5 units per 1 kg body weight given at this stage reduces the content of these constituents in the urine.

In dog No. 1, for instance, the total nitrogen content before injection of ACTH was 4.2 g in 24 hours, after injection of ACTH it became 2.4 g in 24 hours; the amino acid nitrogen content before injection of ACTH was 128 mg in 24 hours and after injection of ACTH it fell to 49 mg in 24 hours; the level of creatine was 0.50 g

TABLE 2

The Effect of Repeated Injection of ACTH (5 units per 1 kg body weight) on Constituents of the Blood (in mg %)

Dog No.	Sugar			Cholesterol			Ketone bodies			Residual nitrogen			Aminoacid nitrogen		
	days after injection of ACTH														
	0	5-8	10-8	0	5-8	10-8	0	5-8	10-8	0	5-8	10-8	0	5-8	10-8
3	75	84	77	137	95	94	—	—	—	29	46	40	—	—	—
5	—	—	—	140	93	89	7.5	6.0	4.4	32	44	37	8.4	12.0	7.6
6	—	—	—	93	110	96	5.8	7.0	1.3	43	34	35	12.3	10.2	8.7

before injection and 0.16 g in 24 hours after injection of ACTH; the creatinine content before the experiment was 0.18 g and after injection of ACTH it fell to 0.10 g in 24 hours.

In precisely the same way, when the excretion of nitrogenous constituents of the urine is increased after administration of thyroidin in the diet, injection of ACTH lowers the total nitrogen, amino acid nitrogen, creatinine and creatine contents of the urine. In dog No. 2, for instance, the total nitrogen excreted in the urine before injection of ACTH was 6.5 g, and after injection of ACTH in the above dose it was 2.3 g in 24 hours; the amino acid nitrogen excreted before injection of ACTH was 116 mg, and after injection of ACTH — 1.27 mg in 24 hours; the creative content before injection of ACTH was 1.27 and after injection — 0.40 g in 24 hours; the corresponding creatinine levels were — before injection of ACTH, 1.02 g and after injection of ACTH, 0.31 g in 24 hours.

The results given show that ACTH and consequently, the glucocorticoids of the adrenal cortex in a determined dose play an essential role in physiological regulation by normalizing abnormal metabolic processes. This adaptive effect of ACTH is evidently brought about by appropriate functional changes in the adrenal cortex, in so far as the function of this organ can be judged by the excretion of neutral 17-ketosteroids in the urine. Parallel investigations of the excretion of 17-ketosteroids in the urine gave the following results: in a series of experiments in which the level of excretion (for the particular animal) of 17-ketosteroids in the urine was low or average, injection of ACTH in a dose of 5-15 units per 1 kg body weight raised the level of excretion. If the initial level of excretion of 17-ketosteroids in the particular animal was high, injection of ACTH either did not change this level, or lowered it (Table 3, dogs Nos. 6 and 3).

TABLE 3

The Effect of a Single Injection of ACTH on the Excretion of 17-Ketosteroids in the Urine

Dog no.	Dose of ACTH, in units per 1 kg body weight	17-ketosteroids, mg:/24 hr	
		before injection of ACTH	after injection of ACTH
8	5	3.7	5.2
8	5	3.3	4.4
8	5	8.4	9.1
3	5	8.6	5.0
3	5	3.0	4.0
6	15	1.3	2.8
6	15	4.4	1.6

The initial functional state of the adrenal cortex thus determines the character of its response to the injection of ACTH and hence the direction of its influence on metabolism. The action of ACTH on the function of the adrenal cortex does not consist only of stimulation, but when certain doses of ACTH are given in certain conditions, of the correction or normalization of this function.

SUMMARY

Examination of the fat-carbohydrate and nitrogen metabolism in the blood and urine of dogs demonstrated that the initial condition of the adrenal cortex may determine the character of response of adrenal cortex to the administration of ACTH. The effect of ACTH on the function of adrenal cortex, probably consists not only in its stimulation but also in normalization (in definite conditions and with certain doses of ACTH).

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DOES THIAMIN DEHYDROGENASE EXIST?

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Previously published experimental results [5] have compelled us to regard as erroneous the statements of A. A. Titaev [1-3] on the existence of thiamin dehydrogenase, an enzyme acting as a specific catalyst of the transfer of hydrogen from thiamin to oxidized forms of adrenalin. According to A. A. Titaev, a measure of the thiamin dehydrogenase reaction is the amount of thiochrome formed in a medium containing thiamin, oxidized adrenalin, a buffer system and, as enzyme, an extract of the hypophysis, thyroid gland or blood plasma. According to A. A. Titaev also, liver and kidney contain a factor which specifically depresses the thiamin dehydrogenase reaction.

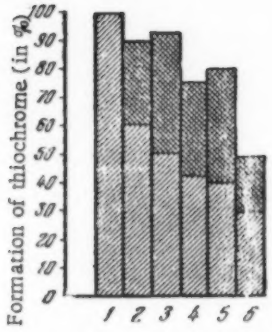
In none of the experiments previously carried out [5] have we been able to find a thiamin dehydrogenase action in any of the preparations tested. The quantity of thiochrome formed in the control tubes always exceeded that in the experimental tubes. We can agree with A. A. Titaev's conclusions only if we pay no attention to the quantity of thiochrome formed in the control tubes. In that case it may be said that perceptibly more thiochrome is formed in the tubes with extract of hypophysis or blood plasma than in the tubes with extracts of tissue homogenates of either liver or kidney.

It is, however, clear that since more thiochrome is formed in the controls than in any variant of the experiment, the relatively high thiochrome formation in the tubes with plasma and hypophysis cannot be regarded as the result of activation of thiochrome formation or as the manifest action of thiamin dehydrogenase. All the tissue preparations depress the "thiamine dehydrogenase" reaction but to a varying degree, and plasma and hypophysis less than liver. It may be thought that the action of depressing the oxidation of thiamin depends to some extent on the intensity of the thiamin metabolism in the tissues, the oxidation-reduction potential of the tissue, and so on.

Other facts which oppose the idea of enzymic catalysis of the formation of thiochrome from thiamin were the results of our experiments in which the "enzyme" preparations were preliminarily heated to 100°C. It was found [5] that this heating does not inhibit thiochrome formation in the tubes but, on the contrary, it increases it almost to the level of its formation in the control tubes and over the level in the unheated tubes.

Having obtained such unexpected results, we returned to A. A. Titaev and, following his instructions, varied the experimental conditions, in particular by altering the concentrations of the reagents. We were still unable to obtain a positive result — evidence of the existence of thiamin dehydrogenase.

After the publication of these facts [5], A. A. Titaev took up his pen [4] in sharp criticism of our work, asserting that our results were negative because we did not observe the necessary experimental conditions, that the distilled water was not pure enough (?) and so on. In fact A. A. Titaev did not reply to any of the questions asked in our paper and gave no explanation at all of the increase in thiochrome formation after heating the enzyme extracts. Being in no doubt of the correctness of our first results so far as conforming to the experimental conditions described by A. A. Titaev was concerned, (especially because we followed his personal instructions), we deemed it necessary to subject this question of the existence of thiamin dehydrogenase to fresh experimental proof.



Formation of thiochrome in several variants of experiments during incubation for 20 hours at 37°C. The extra thiochrome formed in the tubes preliminarily heated to 100°C for 15 minutes is shown cross-hatched. The ordinate axis indicates the amount of thiochrome as a percentage of the control.
1 – control; 2 – hypophysis; 3 – blood plasma; 4 – thyroid gland; 5 – kidney; 6 – liver.

In new experiments we attempted to take into consideration all the existing remarks of A. A. Titaev, and we used a fundamentally different method: instead of ordinary thiamine and determination of thiochrome by a fluorescent method, we used thiamin labeled with radioactive sulfur S^{35} and determined the amount of thiochrome by the radioactivity of butanol extracts or of the appropriate areas of paper chromatograms [6].

EXPERIMENTAL METHOD

In the experiments we used male white rats weighing about 200 g. The animals were killed by exsanguination after exposure of the jugular vein under ether anesthesia. We used water, doubly distilled in glass apparatus. Into each of a row of tubes was poured 0.6 ml of 1/15 M phosphate buffer (pH = 7.2), 0.2 ml of a solution of S^{35}-thiamin (360 μg and 5 μc in 1 ml), 0.2 ml of adrenalin solution (230 μg/ml), and then either 0.05 ml of a 0.9% solium chloride solution (control) or 0.05 ml of freshly prepared homogenate of an organ or of blood plasma (in preparing the homogenates to one part by weight of tissue was added 2 parts of 0.9% sodium chloride solution). Next, to the tubes was added one drop of chloroform as an antiseptic, the tubes were sealed with corks and incubated at 37°C for 20-24 hours. In another variant of the experiment thiamin and adrenalin were added to the tissue homogenate or plasma in phosphate buffer after preliminary heating on a boiling water bath and subsequent cooling.

After incubation, 0.05 ml of the contents of each tube was transferred to paper and separated chromatographically into thiamin and its conversion products by means of a mixture of butanol, methanol, formic acid and water in proportions of 110 : 80 : 15 : 10 [6]. Parallel with the chromatographic analysis the amount of thiochrome in the tubes was determined by the radioactivity of a butanol extract. The extraction was carried out by shaking up 0.5 ml of the incubated mixture with 1 ml of butanol. The tubes were centrifuged and a measured volume of the butanol extract dried on an aluminum foil dish. The radioactivity was determined by means of an end-type radiation counter with a mica window of thickness 1.9 mg/cm^2.

EXPERIMENTAL RESULTS

Only two substances – thiamin and thiochrome – could be detected on the chromatograms. Quantitative analysis of the chromatograms showed that the greatest amount of thiochrome is formed in the control tubes and that thiochrome formation in the experimental tubes is increased after preliminary heating. Similar results were given also by direct determination of the thiochrome by the radioactivity of the butanol extract.

A typical experimental result is shown in the Fig., from which it is seen in the first place that the most thiochrome is formed in the control tubes – in which about 20% of the thiamin is converted into thiochrome. The thiochrome content of the control tubes is taken as 100%.

In all the tubes containing organ homogenates or blood plasma, the thiochrome formation was significantly less than in the control tubes. Thus in the tubes with hypophysis the amount of thiochrome was 60%, in tubes with plasma – 50%, with thyroid gland – 43%, with kidney homogenate – 39% and with liver homogenate – 30%.

And so activation of oxidation of thiamin – the presence of a "thiamin dehydrogenase" system – does not appear in any of the variants of the experiment. As seen from the Fig. some activation is observed after preliminary heating of the homogenates.

The experiments confirm the results previously obtained, and we may state once again that after following A. A. Titaev's experimental procedure we cannot accept as correct his views on the existence of an enzyme thiamin dehydrogenase. All the organs and tissues tested show antithiamin dehydrogenase activity, i.e. prevent oxidation of thiamin into thiochrome. This antithiamin dehydrogenase activity is reduced if the tissue preparations are heated to 100°C.

SUMMARY

The results of experiments on the study of the reaction between thiamine and certain forms of adrenalin leading to formation of thiochromine are presented. Contrary to the views of A. A. Titaev this reaction is not catalysed by a specific enzyme "thiamindehydrase". The quantity of thiochrome formed in control tests is always greater than that in the test with the extracts or homogenates of various tissues. The tissue extracts and homogenates do not activate, but on the contrary depress the reaction. This depression is found to be considerably decreased after preliminary thermal denaturation of the preparations.

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THE ABILITY OF THE BODY TO RETAIN AN INDIFFERENT GAS IN A STATE OF SUPERSATURATION

COMMUNICATION I. THE RELATIONSHIP BETWEEN THE PERMISSIBLE SUPERSATURATION AND CERTAIN BIOPHYSICAL AND BIOCHEMICAL INDICES IN THE BLOOD AND THE HYDROPHILIA OF THE SKIN

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With a fall in the environmental pressure of man, sorption of indifferent gases into the body tissues is reduced. When the rate of fall of pressure does not exceed a certain value, the body frees itself from excess of gas by a process of molecular diffusion. If this condition is not satisfied the indifferent gas is released directly into the tissues in a phase of bubble formation. The development of these gas bubbles when the environmental pressure falls is the fundamental cause of decompression sickness. Persistent bubbles begin to form only when there is a definite discrepancy between the tension of the gas in the body and the general pressure. The value of this pressure difference is given the name of the permissible supersaturation (PS).

From work by several authors [5, 13] it may be assumed that the PS varies from one individual to another. The object of the present investigation was to try to explain the mechanism of these individual differences in the ability of the body to retain an indifferent gas in a state of supersaturation.

We could find no reports in the literature which were directly concerned with the solution of this problem. In carrying out our investigation we had, therefore, to start from a predetermined hypothesis based mainly on results described by M. P. Brestkin [4], American workers [12], D. N. Nasonov and V. Ya. Aleksandrov [7], N. V. Lazarev [6] and M. I. Yakobson [11].

In the present work we investigated the relationship between the individual ability of the body to withstand a definite pressure difference without developing decompression sickness and the following blood indices: the serum protein content, the hemoglobin, the erythrocyte sedimentation rate, the mean corpuscular volume, and also the hydrophilia of the skin.

EXPERIMENTAL METHOD

Experiments were carried out on 7 male dogs of average nutritional level kept on a strict intake of water and food. Twice a week 4 - 4.5 ml of blood was taken from the anterolateral metatarsal vein of each dog, the body temperature taken and a test of the hydrophilia of the skin performed [8]. After this test the dog was placed for 4 hours in a compression chamber at normal atmospheric pressure; occasionally the animal was weighed and its pulse rate taken before being placed in the chamber.

The mean corpuscular volume of the blood was determined with the hematocrit and the hemoglobin by a colorimetric method [3]. A small portion of the blood taken was mixed with ammonium and potassium oxalate in order to obtain plasma and from the rest of the blood, serum was obtained by a method ensuring no contamination with hemoglobin [2]. The total protein content of the plasma was estimated by the biuret reaction and in the serum – the total protein content and the albumin content after precipitation of the globulins with saturated

TABLE 1

Values of the Permissible Supersaturation of the Body with Nitrogen in the Experimental Dogs

V'yunok		Pestryi		Polkan		Mars		Bobik		Tsyganok		Atlas	
date	PS	date	PS	date	PS	date	PS	date	PS	date	PS	date	PS
30/V 1956 r.	1.72	27/X 1956 r.	1.72	12/VI 1956 r.	1.56	8/V 1956 r.	1.4	24/IX 1956 r.	1.4	19/XI 1956 r.	1.24	10/V 1956 r.	1.08
8/VI	1.72	23/XI	1.72	20/VI	1.56	22/V	1.4	24/X	1.4			24/V	1.08
18/IX	2.04	7/I 1957 r.	1.72	13/IX	1.56	6/IX	1.4	26/XI	1.4			5/IX	1.08
28/IX	2.04			30/XI	1.56	29/X	1.56						
14/I 1957 r.	2.04					8/I 1957 r.							

sodium sulfate solution [15]. In addition two precipitation reactions were carried out with the serum: zinc sulfate [10] and coagulating [1]. For the colorimetric measurements we used the FÉK-M colorimeter.

After the preliminary experiments had given clear results we proceeded to measure the PS by the method of I. I. Savichev and A. N. Bukharin [5]. For this purpose, after taking the blood and carrying out the test of hydrophilia of the skin, we placed the dog in the chamber and raised the pressure to 2.4 absolute atmospheres. At the end of 4 hours the pressure was lowered to atmospheric within 50-60 sec. If signs of decompression sickness appeared, a therapeutic recompression was carried out. In the absence of signs of the sickness, after a minimum of two days the dog was again placed in the chamber, this time at a pressure of 0.2 absolute atmos above the previous. This continued until after release from any pressure decompression sickness developed. The value of the PS was taken to be the maximum pressure which did not cause the sickness. The PS was expressed by the formula $\Delta_p = PN_2 - P_A$, where PN_2 is the nitrogen pressure in the tissues after exposure for 4 hours, in practice equal to the partial pressure of N_2 in this total pressure [14]; P_A is the atmospheric pressure, equal to 1 absolute atmos.

The results of measurement of the blood indices were analysed statistically by the rules generally accepted in experimental work [9].

EXPERIMENTAL RESULTS

As seen from Table 1, over a period of 3-8 months small variations in the PS were observed in only 2 experimental dogs.

In order to fulfil the main task of the investigation, we carried out 194 experiments in 16 series. The experimental results are shown in Table 2. In this table the significant arithmetic mean values of all indices are given, obtained in accordance with the variation of the index from 27 to 3 separate values. In the two lower lines of the table are indicated the statistical criteria of the relationship, in this case also only when of proved significance.

It follows from the results in Table 2 that there is a close statistical relationship between the PS and the protein composition of the serum: the higher the albumin content and the lower the globulin content, the greater the degree of supersaturation which the dog as a rule is able to withstand. This relationship is seen particularly clearly from a comparison of the PS and the albumin-globulin ratio (A/G). This relationship between the PS and A/G is shown graphically in the Fig.*

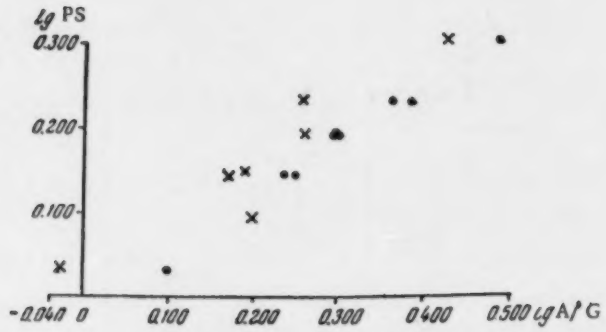
The character of distribution of the points on the graph suggests the presence of an almost proportionate relationship between the PS and the value of the A/G. It is noteworthy that the points which relate to the observations made in the autumn (x) are situated to the left of those relating to the winter and spring(●). Evidently in the autumn some factor acts on the body which causes the PS to increase without

* The graph is drawn to a logarithmic scale in order to reduce its size.

TABLE 2

The Relationship between the PS and Various Biophysical and Biochemical Indices

Name of dog	Age (in years)	Serial number of experiments	PS, atmos.	Body weight, kg	Body temperature, °C	Pulse rate, per minute	Mean corpuscular volume, %	Plasma proteins, g%	Serum proteins, g%	Albumins, g%	Globulins, g%	Albumin/Globulin ratio	Zinc sulfate reaction, extinction	Coagulation test (highest dilution with coagulation)	Hemoglobin, extinction	ESR, mm/hr	Time of absorption of physiological saline, minutes
V'yunok	1—2	1	1.72	19.4	39.0°	—	40.4	5.62	5.40	3.72	1.62	2.32	—	0.31	0.418	3.0	41
		2	2.04	18.8	38.8°	88	42.3	5.72	5.55	4.05	1.53	2.68	0.035	0.21	0.516	2.1	29
		3	2.04	20.6	38.9°	89	44.5	5.82	5.72	4.30	1.40	3.09	0.020	—	—	—	33
Pestryi	3—4	4	1.72	21.6	38.2°	100	52.6	6.06	6.01	3.85	2.14	1.82	0.026	—	—	—	40
		5	1.72	22.1	38.2°	84	51.2	—	6.28	4.46	1.82	2.44	0.024	—	—	—	46
Polkan	1—2	6	1.56	29.8	38.4°	—	41.5	6.42	6.11	4.11	2.01	2.10	—	0.26	—	—	44
		7	1.56	28.4	38.8°	84	42.7	6.01	5.73	3.64	1.97	1.82	0.046	0.22	—	—	31
		8	1.56	26.4	38.5°	63	45.3	6.43	6.25	4.15	2.10	2.00	0.060	—	—	—	34
Mars	4—6	9	1.40	24.3	38.5°	—	43.8	5.75	5.54	3.54	1.97	1.79	—	0.25	0.450	3.0	64
		10	1.40	24.4	—	91	42.7	5.91	5.66	3.34	2.29	1.49	0.034	—	—	—	34
		11	1.56	25.0	—	75	42.7	6.11	5.99	4.06	1.94	2.10	0.028	—	—	—	43
Tsyganok	2—3	12	1.24	19.2	38.8°	79	43.4	5.93	5.67	3.47	2.20	1.59	0.072	—	—	—	25
Bobik	5—7	13	1.40	21.4	38.3°	105	48.9	6.36	6.03	3.67	2.41	1.55	0.075	—	—	—	40
		14	1.40	20.1	38.2°	92	44.4	6.10	6.05	3.85	2.21	1.73	0.049	—	—	—	45
Atlas	3—4	15	1.08	24.3	38.6°	—	45.6	6.18	5.80	3.20	2.61	1.26	—	0.30	0.446	3.0	64
		16	1.08	24.2	38.5°	80	46.7	6.34	6.04	2.91	3.13	0.93	0.187	0.24	0.508	7.0	54
Criteria of the relationship between the PS and the given indices	Coefficient of correlation, r	—	—	—	—	—	—	—	+0.80	—0.89	+0.94	—	—	—	—	—0.50
	Correlation ratio η	—	0.89	—	0.89	—	—	—	—	—	—	0.95	—	—	—	—



Relationship between the value of the permissible supersaturation of the body with nitrogen and the value of the albumin-globulin ratio of the blood serum.
x) Observations made in the autumn; ●) observations made in the winter and spring.

at the same time affecting the composition of the blood proteins.

The correlation between the PS and the composition of the serum proteins is confirmed both by the presence of a relationship between the PS and the zinc sulfate test, although in this case the relationship is not linear but curvilinear, in the form of a hyperbola.

A curvilinear relationship was also found between the PS on the one hand and the body weight and pulse rate on the other. The curve in this case was S-shaped. It is difficult to understand the meaning of this phenomenon without further investigation.

The relationship between the PS and the hydrophilia of the skin merits attention. The closeness of this relationship is considerably less than between the PS and the protein composition of the serum, but the shape of the graph here is quite definite and is linear in character: The higher the PS the greater the hydrophilia.

Hence the physiological indices most closely connected with the PS are the albumin and globulin content of the blood serum.

It is interesting that in cases when variations in the PS were observed, as for instance in the dogs Mars and V'yunok, the increase in the PS was accompanied by an increase in the mean value of A/G. Consequently here also we find confirmation of the relationships which have been discovered.

On the whole the results given in this paper demonstrate the existence of a well defined relationship between the ability of the body to retain nitrogen in a supersaturated state, the protein composition of the blood and the water metabolism. Evidently differences in the colloidal properties of the blood and other fluid tissues are the factor which to a large extent determines the value of the permissible supersaturation of the body with an indifferent gas.

SUMMARY

The relationship of the ability of the body to retain nitrogen in condition of oversaturation to the qualitative pecularities of the blood proteins and to skin hydrophilism was demonstrated in chronic experiments on dogs.

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THE COMBINED ACTION OF THE SPLEEN AND OTHER ORGANS

COMMUNICATION II. THE RATE OF RENEWAL OF PROTEINS, THE SYNTHESIS OF UREA AND THE CONTENT OF THE ANTIANEMIC FACTOR IN THE LIVER OF WHITE RATS WITH EXPERIMENTAL SPLENOPATHY

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The problem of the splenogenic nature of certain chronic diseases of the liver cannot yet be regarded as finally settled [2-4]. The great practical importance of this problem in clinical practice justifies experimental research in the direction of studying the correlation of liver and spleen in pathological conditions.

Our report is based on the results of investigation of certain indices of liver activity in experimental splenopathy. We determined the rate of renewal of proteins, the formation of urea and the content of antianemic factor in the liver of white rats with splenomegaly caused by methylcellulose.

EXPERIMENTAL METHOD

The experiment was carried out on white rats weighing 120-160 g kept on a balanced diet. Splenomegaly was induced by intraperitoneal injection by methylcellulose for 15 weeks (2 ml of a 2.5% solution twice a week). In some animals the spleen was removed 10 days before the injections of methylcellulose were started and to prevent bartonelliasis, novarsenol was injected in a dose of 0.3 mg per 100 g body weight. As a control intact rats were used on which a spurious splenectomy was performed (laparotomy without removal of the spleen, and injection of novarsenol), followed by injections of methylcellulose, and splenectomized rats not receiving subsequent injections of methylcellulose but receiving novarsenol.

The rate of renewal of protein was studied by injecting the experimental animals subcutaneously with radioactive methionine (labeled with S^{35}, in a dose of 5000 impulses/min per 1 g body weight of the animal) dissolved in physiological saline. The rats were starved for 6 hours before the injection of methionine to prevent dilution of the label with the methionine of the food. At different intervals after injection of the labeled amino acid, animals were killed by decapitation, the liver extracted, ground up finely, the proteins precipitated with a 10% solution of trichloroacetic acid, washed twice in a centrifuge with 5% trichloroacetic acid solution and treated with 10 times its volume of alcohol to extract the lipids; the residue was then washed twice with an alcohol-ether mixture (1:1) and twice with ether, and then dried in an incubator for 24 hours. After drying, the protein was rubbed into a fine powder, and samples of the dried protein weighing 10 mg were taken for estimation of its radioactivity with a Geiger-Müller counter.

In order to determine the urea formation in the liver, sections of the organ were washed with a bicarbonate buffer solution (pH = 7.4) and placed in a vessel containing 6 ml of the same buffer. To this solution was added a solution of ammonium chloride in volume to produce a final concentration of 1/100 M (17 mg% of ammonia). In one series of experiments glucose was also added to the solution to determine its stimulating action on the process of urea formation (200 mg per tube). The vessels were filled with a gas mixture consisting of 5.6% CO_2

TABLE 1

The Effect of Experimental Splenomegaly due to Methylcellulose on the Inclusion of Radioactive Methionine in the Liver Proteins in White Rats

Group No. of rats	Group characteristics	Number of animals	Radioactivity of 10 mg of liver protein as a % of that injected per 1 g body weight
1	Intact	15	4.1 ± 0.7
2	Splenomegaly (methylcellulose for 15 weeks)	15	2.2 ± 0.5
3	Splenectomy + methylcellulose for 15 weeks	15	3.9 ± 0.8
4	Spurious splenectomy + methylcellulose for 15 weeks	15	2.5 ± 0.8
5	Splenectomy (examination 3 weeks after operation)	15	3.2 ± 0.7
6	Splenectomy (examination 3 months after operation)	10	4.2 ± 0.4
7	Spurious splenectomy (examination 3 weeks after operation)	10	4.0 ± 0.8

Note: In the table are shown mean values and mean square deviations from these for each group.

TABLE 2

The Effect of Experimental Splenomegaly due to Methylcellulose on Urea Formation in the Liver

Group No. of Rats	Group characteristics	Number of animals	Urea formation in the liver in μM of nitrogen per 1 g of tissue	
			without addition of glucose	with addition of glucose
1	Intact	15	62.8 ± 3.5	94.0 ± 7.1
2	Splenomegaly (methylcellulose for 15 weeks)	15	15.6 ± 5.9	18.7 ± 4.4
3	Splenectomy + methylcellulose for 15 weeks)	15	58.6 ± 7.2	82.7 ± 9.9
4	Spurious splenectomy + methylcellulose for 15 weeks)	8	19.7 ± 5.8	18.5 ± 7.2
5	Splenectomy (examination 3 weeks after operation)	10	66.5 ± 5.2	88.1 ± 5.2

and 94.4% O_2, and were placed in a water agitator for 2 hours at 38°C. After this the proteins were precipitated with a 10% solution of trichloroacetic acid, and the fall in the ammonia content of the filtrate and the amount of urea synthesized estimated by Kreb's method [5].

In order to estimate the content of antianemic factor in the liver, samples of the organ weighing 250 mg were mixed with 6 ml of Ringer-Locke solution. After standing for 30 minutes in the cold, the extract was treated

TABLE 3

The Effect of Experimental Splenomegaly due to Methylcellulose on the Content of Antianemic Factor in the Liver of White Rats

Group No. of rats	Group characteristics	Number of animals	% change in the reticulocyte count in recipient rats after injection of ultrafiltrate of liver of experimental rats
1	Intact	15	+ 178.3 ± 19.1
2	Splenomegaly (methylcellulose for 15 weeks)	15	+ 41.5 ± 14.8
3	Splenectomy + methylcellulose for 15 weeks)	15	+ 156.8 ± 20.9
4	Spurious splenectomy + methylcellulose for 15 weeks)	10	+ 34.8 ± 8.2
5	Splenectomy (examination 3 weeks after operation)	10	+ 182.1 ± 25.4

by ultrafiltration to remove the proteins; absence of protein from the ultrafiltrate was verified by the reaction with sulfosalicylic acid. The ultrafiltrate was injected subcutaneously in a volume of 2 ml into intact rats in which reticulocyte counts were made on several occasions before and after the injection of the ultrafiltrate of the liver of the experimental animals. The reticulocytes were counted in relation to 1000 red cells. An increase was regarded as specific if it reached 150-200% of the mean value before injection of ultrafiltrate and appeared not later than the 5th day after injection.

This particular method was tried out in rats [1].

EXPERIMENTAL RESULTS

The experimental results are shown in Tables 1-3.

From the data in Table 1 it can be seen that in experimental splenomegaly due to methylcellulose there is a marked fall in the rate of inclusion of radioactive methionine in the protein of the liver; this shows a fall in the rate of renewal of proteins in the liver. Splenectomy itself, at early periods after removal of the spleen, also leads to some degree of slowing (far less pronounced than after splenectomy, it is true) of the rate of renewal of protein, but this effect of splenectomy is quickly compensated and 3 months after splenectomy the inclusion of radioactive methionine in the liver protein of the splenectomized animals was indistinguishable from that in the controls. The fact that injection of cellulose into splenectomized animals does not change the rate of renewal of protein in the liver is an indication of the importance of splenectomy as such in the development of retardation of the inclusion of radioactive methionine in the liver proteins.

From Table 2 it can be seen that urea formation in the liver during experimental splenomegaly is disturbed and the leading role in this disturbance belongs to the spleen which has undergone pathological changes and not to the methylcellulose itself. It is interesting that the addition of glucose to the liver slices from the animals with splenomegaly does not improve urea formation; this suggests some disturbance of the enzyme systems catalyzing the process of urea formation in the liver. Removal of the spleen, even in early periods after the operation, does not affect the urea-forming function of the liver.

Table 3 shows a clear reduction in the content of antianemic factor in the liver of rats with experimental splenomegaly, as judged by the reticulocyte reaction of recipient rats. In this phenomenon also it is not methylcellulose as such but the splenomegaly which it causes which plays the leading part, as may be seen from Table 3.

The results of this last series of experiments, summarized in Table 3, appear to us to shed light on some of the mechanisms of the so-called splenic cytopenias, and particularly anemia, occurring in various diseases accompanied by enlargement of and pathological changes in the spleen.

The results of our experiments indicate a close link between the spleen and liver in pathological conditions and a marked influence of the pathological spleen on the liver function.

SUMMARY

In experimental splenomegaly of white rats caused by the intraperitoneal injection of methylcellulose the velocity of protein renovation is found to be decreased in the liver (determined by the test of radioactive methionine incorporation into the liver proteins). The urea-forming function of the liver is also disturbed (tested of the urea synthesis by the liver sections). Experiments demonstrated that the pathological enlargement of the spleen with the disturbance of its functions reduces the antianemic factor content of the liver. This may to a certain extent explain the anemia occurring in various diseases connected with splenomegaly.

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PHARMACOLOGY

BIOCHEMICAL METHODS OF REMOVAL OF THE BLOCKING ACTION OF METHYLENE BLUE ON THE VAGUS NERVE

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The transmission of excitation along nerves is known to be disturbed by chemical agents acting on any of the links in the chain of metabolism. One of the substances which blocks the transmission of nervous excitation (or inhibition) from the vagus nerve to the heart is methylene blue.

Koskowski and Maigre [9] observed that methylene blue causes a paralytic condition of the vagus nerve, and M. L. Belen'kii demonstrated that methylene blue inhibits the action of acetylcholine on the frog's heart. I. A. Keder-Stepanova [2] showed that methylene blue in a concentration of $2 \cdot 10^{-6}$ g/ml causes reversible inhibition of the action of the vagus nerve on the frog's heart but large concentrations of this substance have an irreversible effect. Kh. S. Koshtoyants and S. S. Mogoras [4], in experiments on frogs, demonstrated that the reflex inhibition of the activity of the heart may also be removed by the action of methylene blue on the heart. It was shown in work by É. A. Kyandzhuntseva [6] that methylene blue inhibits the action of the vagus nerve on the heart of warm-blooded animals also.

A large number of facts has recently been accumulated showing the importance of sulfhydril groups in the transmission of nervous excitation, in particular in relation to the influence of the vagus nerve on the heart [3, 5, 7, 8].

Ks. S. Koshtoyants has postulated that the effect of methylene blue may be explained by the interaction between methylene blue and the sulfhydril groups. This hypothesis was based on biochemical data which indicated that methylene blue, as a hydrogen acceptor, may take part in the process of conversion of SH-groups into SS-groups. In order to verify this hypothesis experimentally, we carried out experiments to discover whether it is possible to restore the inhibiting action of the vagus nerve and also of acetylcholine on the cardiac muscle, blocked by methylene blue, by the action of substances such as SH-glutathione and hydrogen sulfide. In the present report we describe the results of these experiments.

EXPERIMENTAL METHOD

Experiments were carried out on frogs' hearts, isolated by Straub's method and perfused through the conus arteriosus. In cases where the vagus nerve was to be stimulated, the heart remained connected to the medulla oblongata through the vagosympathetic trunk; the vagus nerve center in the medulla was stimulated by means of silver electrodes. The experiments were recorded by means of a mechanical recording system and by electrocardiograms of the heart.

The action of the following substances was tested: 1) methylene blue – $2 \cdot 10^{-3}$; $2 \cdot 10^{-4}$; $2 \cdot 10^{-5}$ g/ml; 2) acetylcholine – $1 \cdot 10^{-6}$; 3) reduced glutathione – $1 \cdot 10^{-2}$ g/ml; 4) atropine – $1 \cdot 10^{-5}$; $1 \cdot 10^{-6}$ g/ml; 5) hydrogen sulfide (H_2S).

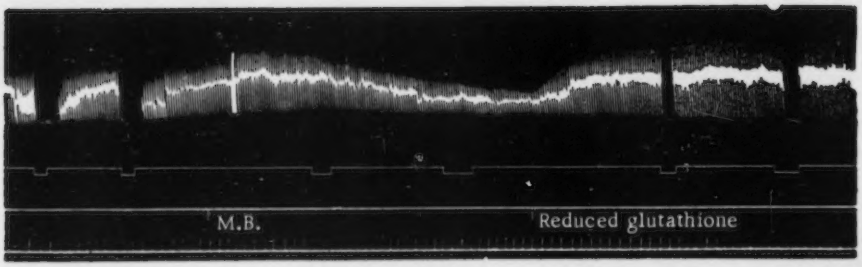

Fig. 1. Restorative action of glutathione on the effect of the vagus nerve after the action of methylene blue.
Significance of the curves (from above down); contractions of the cardiac muscle; stimulus marker; marker of the moment of injection of the substances into the heart (methylene blue $2 \cdot 10^{-4}$, reduced glutathione $1 \cdot 10^{-2}$); time marker (5 seconds).

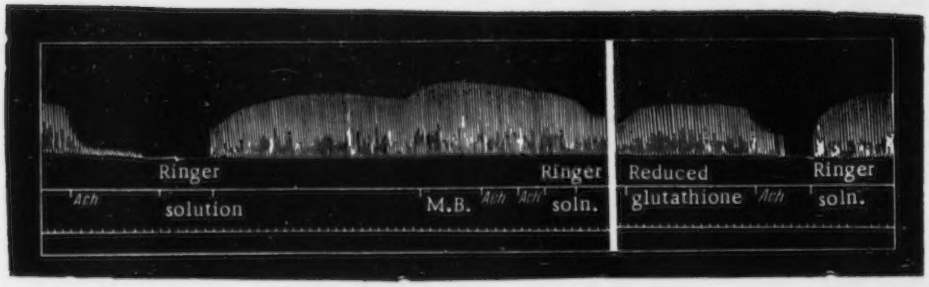

Fig. 2. Restorative action of glutathione on the effect of acetylcholine after the action of methylene blue.
Significance of the curves as in Fig. 1. Acetylcholine — $1 \cdot 10^{-6}$ (0.04 ml) methylene blue — $2 \cdot 10^{-4}$; reduced glutathione — $1 \cdot 10^{-2}$.

The solutions of methylene blue and reduced glutathione were prepared in Ringer solution, and the solutions of acetylcholine and atropine in distilled water. Ringer solution was saturated with H_2S from a Kipp's apparatus. The gas was usually passed through a tube of known diameter for 5-20 sec into 20-25 ml of Ringer solution.

The number of H_2S bubbles passing through a given volume of solution was recorded on the kymogram (H_2S — 10/25). The solutions of hydrogen sulfide and glutathione were freshly prepared on the day of the experiment; the pH of the solutions was from 7.0 to 7.5.

EXPERIMENTAL RESULTS

By investigating the action of methylene blue on the frog's heart we established that this substance, in concentrations of $2 \cdot 10^{-3}$ and $2 \cdot 10^{-4}$, blocks the action of the vagus nerve on the heart after $1\frac{1}{2}$ to $3\frac{1}{2}$ minutes.

Prolonged perfusion of the heart with Ringer solution (for 40-50 minutes) after this may lead to restoration of the effect of stimulation of the vagus nerve center. After the prolonged action of methylene blue on the heart, the inhibiting effect of the vagus nerve cannot be restored by perfusion of the heart with Ringer solution.

Comparison of the mechanographic and electrographic recordings showed that in 80% of the experiments the effect of the vagus nerve on the heart, after the action of methylene blue, is altered and often is completely

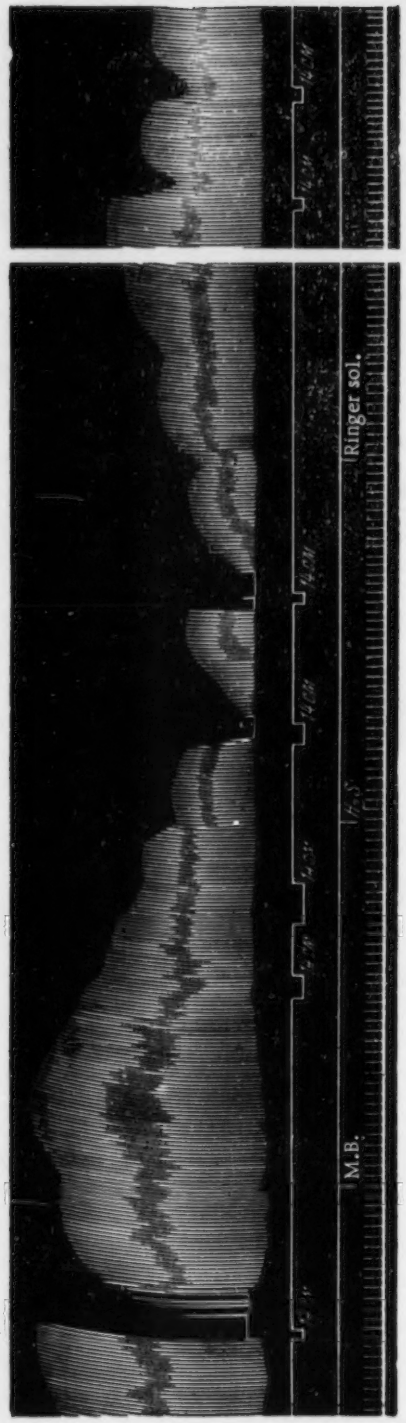

Fig. 3. Restorative action of hydrogen sulfide on the vagus nerve effect. Significance of the curves as in Fig. 1. H_2S – 10/25.

blocked; in 49% of the 80% of experiments an increase in the heart rate was observed in response to stimulation of the vagus nerve center.

As the main criteria of the condition of the heart during the experiments we examined: the change in rhythm during stimulation of the vagus nerve; the change in atrioventricular conduction and also the changes in the form of the electrocardiogram (T wave).

In relation to the findings described above, M. L. Belen'kii pointed out that methylene blue in these concentrations blocked the depressing effect of acetylcholine ($1 \cdot 10^{-6}$).

The effect of acetylcholine was not restored by prolonged perfusion of the heart with Ringer solution.

On the basis of the above-mentioned hypothesis of the possible role of SH-groups in the mechanism of the effect of methylene blue on the action of the vagus nerve (and of acetylcholine), we tried experimentally to restore this action (after the prolonged action of methylene blue) by means of SH-glutathione and H_2S. In normal conditions neither glutathione nor H_2S (in the concentrations which we used) caused any perceptoble change in the rhythmic activity of the heart. Occasionally a slight increase in the amplitude of the cardiac contractions may be observed, which rapidly returned to normal; hydrogen sulfide rarely caused a slight slowing of the rate. The inhibitory effect of stimulation of the vagus nerve was hardly affected by these substances.

After introduction of methylene blue solution into the heart phasic changes were observed: at first an increase in the amplitude of the cardiac contractions, strengthening of the cardiac action and then a decrease in the amplitude of the contractions. After the methylene blue had blocked the action of the vagus nerve and of acetylcholine on the heart, the solution of methylene blue was replaced by a solution of reduced glutathione. As a rule the glutathione caused first normalization of the strength and rate of the cardiac contractions, and secondly restoration of the action of the vagus nerve and acetylcholine blocked by the methylene blue (Figs. 1 and 2).

The electrocardiograph recordings confirmed that acetylcholine causes considerable changes in the electrocardiogram: in our experiments these were most clearly shown in the shape of the T wave and also by the shortening and almost complete disappearance of the S-T interval. Under the action of methylene blue the T wave was the most affected (it was flattened, sometimes inverted); in addition there was a significant lengthening of the S-T interval and a diminution of the

R wave. Against a background of methylene blue, acetylcholine caused none of the changes in the electrocardiogram that are characteristic of the action of this drug. On the addition of reduced glutathione (after the action of methylene blue) the electrocardiogram gradually returned to normal (the S-T interval diminished, the R wave and more particularly the T wave voltage increased) and at the same time, against this new background created by the glutathione, addition of acetylcholine caused its characteristic, normal changes in the electrocardiogram which did not appear when acetylcholine was added in conjunction with methylene blue.

Our experiments showed that hydrogen sulfide and reduced glutathione equally abolish the effect of methylene blue and restore the action of the vagus nerve and of acetylcholine on the heart (Fig. 3). When the action of hydrogen sulfide is brief, its effect is reversible in character: addition of methylene blue solution caused the action of the vagus nerve to be blocked; it was restored by perfusion of the heart with Ringer solution saturated with H_2S, and when this was replaced by fresh Ringer solution, marked weakening or disappearance of the vagus effect was again observed. Such a competitive relationship between methylene blue and hydrogen sulfide is probably connected with a transient action of the latter, which can only permit partial and insignificant restoration of SH-groups.

A special series of experiments was devoted to the comparative analysis of the action of methylene blue and atropine (as agents inhibiting the action of the vagus nerve on the heart), in order to discover the role of SH-groups in either case. The experiments showed that whereas after the action of the vagus nerve has been blocked by methylene blue it can be restored by perfusion of the heart with Ringer solution containing hydrogen sulfide, it cannot be restored in the same way after being blocked by atropine.

SUMMARY

The disturbance of the process of "transmission" of the nervous excitation may be caused by chemical agents blocking one or another link of the metabolic process. Methylene blue is one of the substances blocking the transmission of the nervous excitation from the vagus nerve on the heart. Numerous facts point to the important role played by SH groups in the transmission of the nervous excitation. A suggestion was made on the possibility of the reestablishment of the inhibitory effect of the vagus nerve and acetylcholine on frog's heart previously blocked by methylene blue substances containing SH groups (SH-glutathione and by H_2S).

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A REFLEX COMPONENT OF THE MECHANISM OF PRODUCTION OF BLOOD CHANGES BY CHLORTETRACYCLINE (BIOMYCIN)

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During investigations on the effect of chlortetracycline (biomycin) on the physiological processes of the body, carried out in our laboratory, it was found that this antibiotic, when administered orally in certain doses to rabbits, may cause a series of changes in the composition of the blood [8]. The most pronounced changes were in the number of leucocytes — which was increased — and in the proportion of lymphocytes and segmented neutrophils (pseudoeosinophils).

Since chlortetracycline hydrochloride has a stimulating action on organs and tissues, it might be supposed that these changes are the result of both the direct and reflex action of the drug on the hemopoietic organs following stimulation of receptors.

There are several recent reports of work on the nervous regulation of the blood system [1-7, 10-28].

Nevertheless the influence of antibiotics on the blood has received scant attention. G. S. Kan [13] investigated the effect of streptomycin on reflex changes in the composition of the blood resulting from stimulation of interoceptors. This author found that streptomycin depresses leucocytosis due to stimulation of chemoreceptors. No investigations of a similar type have been carried out with chlortetracycline.

In the present communication we give our findings in regard to the role of stimulation of the receptor apparatus in changes in the composition of the leucocytes after intramuscular injection of chlortetracycline, and of its humoral effect on the composition of the leucocytes.

EXPERIMENTAL METHOD

Experiments were carried out on rabbits. Blood was taken from the marginal vein of the ear and the white cell count and leucocytic formula determined (films were stained by Leishman's method).

The rabbits were starved for 18 hours before each investigation. As a preliminary measure, in each rabbit we investigated the effect of the experimental environment on the composition of the white cells of the blood (background investigation). For this purpose blood was taken after 0.5, 1, 2, 3, 6 and 8 hours and in some experiments — after 10, 24, 48 and 72 hours. Animals showing variations in the white cell count, in the tests at these times, greater than ± 22% were not included in the experiment. The leucocytic formula was more stable in the course of the experiment. The percentage of lymphocytes and segmented granulocytes varied on the average by ± 10%, and that of the other cells by a few per cent (1-3). Within the limits of these variations we could observe no particular regular features in the daily variation of the white cell count or composition.

Chlortetracycline was dissolved in physiological saline in a concentration of 10 mg/ml and injected into the muscles of both thighs in a dose of 25 mg per 1 kg body weight. Blood for examination after the injection of

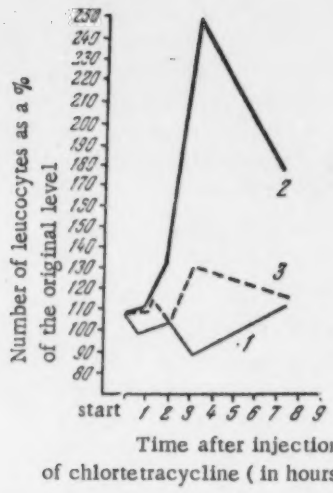

Fig. 1. Changes in the white cell count before and after intramuscular injection of chlortetracycline. 1) White cell count during investigation of the initial background; 2) change in the white cell count after intramuscular injection of 25 mg/kg body weight of chlortetracycline; 3) change in the white cell count after preliminary injection of novocain into the site of injection of chlorotetracycline.

chlortetracycline was taken at the same times as in the background investigation. From 2-3 days after the first investigation, chlortetracycline was again injected in the same dose, but 1-2 minutes before this was done, injections were given at the same points of the right and left thighs of 1.5 ml of a 0.5% solution of novocain.

In view of the fact that the chlortetracycline solution had a pH of 2.9, preliminary control experiments were carried out in which injections were given of physiological saline (5-10 ml) acidified with hydrochloric acid to pH = 2.9 (6 experiments). We also carried out control experiments in the same manner in which 3 ml of a 0.5% solution of novocain was injected intramuscularly (6 experiments). It was established that in each case the daily variations in the composition of the white blood did not exceed those of the background investigation.

EXPERIMENTAL RESULTS

The first series of investigations was carried out on 14 rabbits weighing from 2 to 3 kg; the blood examinations after injection of chlortetracycline or novocain and chlortetracycline continued for 8-10 hours in this series.

After the injections of chlortetracycline all the animals developed leucocytosis. In the majority of the experiments this was apparent between the 1st and 2nd hour after injection of the drug; the white cell count rapidly increased and reached a maximum in 4 rabbits at the 2nd hour, in 6 rabbits at the 3rd hour, and in the remaining 4 at the 5th-6th hour. The increase in the number of leucocytes varied from 30 to 220% of the original count, but in the majority it was between 75 and 220%. After reaching a maximum increase in 9 rabbits, the leucocytosis fell and regained its normal value at the 5th, 6th, 8th or 10th hour. In the 5 rabbits with the greatest leucocytosis, the white cell count after 8-10 hours either remained at a high level (3 rabbits) or was just beginning to fall (2 rabbits). In 3 rabbits, after the white cell count had returned to its original level, it showed a tendency to rise again.

Thus the intramuscular injection of chlortetracycline in a dose of 25 mg/kg regularly caused in all the animals a rapidly developing leucocytosis, the magnitude and duration of which varied in accordance with the individual features of the animals. Different results were found when the chlortetracycline was given after a preliminary injection of novocain at the same point. Of the 14 rabbits, 3 showed a slight leucopenia (to 25-50%) in the first 2-3 hours in place of the leucocytosis, followed by a return of the leucocyte count to its original value after 6-8 hours. In 6 rabbits which had developed a marked leucocytosis in response to the injection of chlortetracycline alone showed a considerably reduced leucocytosis after novocain, and its development was delayed by 1-2 hours. Only in 5 rabbits was the reaction to injection of chlortetracycline unchanged after novocain, although in these experiments the development of maximum leucocytosis was delayed by approximately 1 hour. Furthermore in 3 rabbits the leucocyte count returned to its original value 4-5 hours earlier than after injection of chlortetracycline alone.

Thus in the majority of cases the preliminary injection of novocain either prevents or reduces the degree of leucocytosis, or delays its development and it comes to an end more rapidly (Fig. 1).

The explanation of these findings may be that anesthesia of the nerve endings in the muscles abolishes or reduces the stimulating action of chlortetracycline. Hence it follows that the leucocytosis developing immediately after injection of chlortetracycline includes a reflex component.

In 11 animals of the first series the leucocytic formula was examined in blood taken at the same times as that for the white cell counts. It was found that regular changes took place in the numbers of all forms of leucocytes, mainly in the proportions of the lymphocytes and pseudoeosinophils. In all 11 rabbits a fall was observed

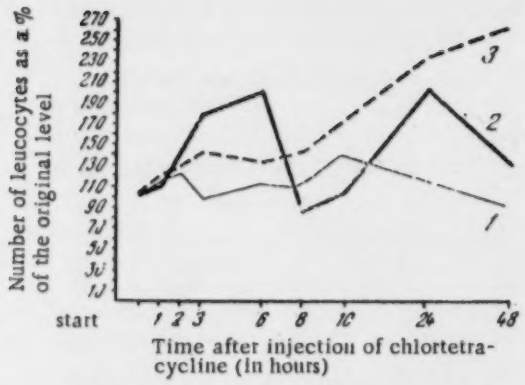

Fig. 2. Changes in the number of leucocytes before and after injection of chlortotracycline for a period of 48 hours. Legend as in Fig. 1.

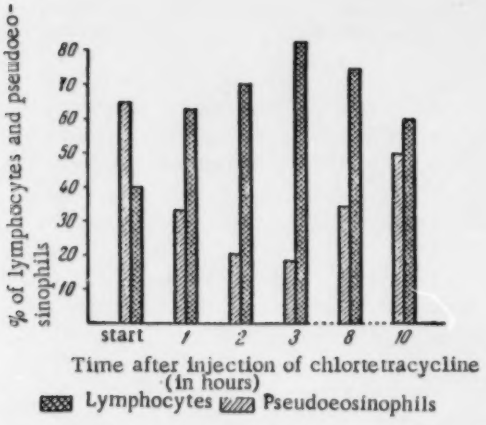

Fig. 3. Changes in the proportions of pseudoeosinophils and lymphocytes after intramuscular injection of chlortetracycline.

in the number of lymphocytes in favor of an increase in the pseudoeosinophils. This fall began from 2-3 hours after injection of the preparation and reached its maximum in 4 rabbits after 3-4 hours and in the rest 5-6 hours. The proportion of lymphocytes fell by 24-44%, on the average by 33.6%, i.e. the absolute lymphocyte count fell by 1½-2 times.

There was an equivalent increase in the content of pseudoeosinophils and no essential change in the other forms of leucocytes.

In 3 rabbits reversal of the changes in the proportions of the leucocytes began from 6-8 hours after the injection of chlortetracycline. In all the other animals this reversal could not be seen even after 10 hours. Consequently these changes were more stable than the leucocytosis.

During the action of chlortetracycline after intramuscular injection of novocain the changes observed in the leucocytic formula were approximately the same as those after injection of chlortetracycline alone. In only 3 rabbits was the fall in the number of lymphocytes less marked than before injection of novocain (by 10-15%).

In the second series of experiments carried out on 8 rabbits, the blood was examined for longer periods after injection of chlortetracycline, as follows: after 1, 2, 3, 6, 8, 10, 24 and 48 and sometimes after 72 hours. In the first 8-10 hours the same results were obtained in the rabbits of this series as in the first series of experiments: in the majority of the animals novocain either prevented or reduced the development of leucocytosis resulting from the action of chlortetracycline.

However the subsequent examinations of the blood picture in this series of experiments showed that after the fall in the leucocytosis after 8-10 hours, the leucocyte count may begin to rise again. Usually from 10-12 hours after the injection of chlortetracycline the white cell count begins to rise again, and reaches a maximum after 24-48 hours. The white cell count is increased by 50-150% over its original value; at the 48th-72nd hour the leucocytosis begins to fall, but it mostly does not regain its original level. It was found that the preliminary injection of novocain does not affect this secondary leucocytosis (Fig. 2).

Despite the small number of animals in this series, the fact that a secondary leucocytosis appears and that it is unaffected by injection of novocain cannot be regarded as accidental, since in the first series of experiments we also observed in some rabbits a tendency for the white cell count to rise again after 10-12 hours.

In the second series of experiments we investigated the leucocytic formula of all the animals in blood taken at the same times. The same changes were found as in the animals of the first series, i.e. a relative fall in the lymphocytes and rise in the pseudoeosinophils (Fig. 3). Their proportions were restored only after 24 to 48 hours or even later, independently of any previous injection of novocain.

The results obtained thus indicate that the leucocytosis arising in animals after intramuscular injection of chlortetracycline is brought about in two ways: in the first few hours it is the result of a reflex act from stimulation

of the receptors of the muscle tissue; later, as absorption of chlortetracycline takes place, it evidently causes a second wave of leucocytosis by a humoral mechanism, and this is maintained much longer than the first.

Chlortetracycline also produces changes in the proportions of lymphocytes and segmented neutrophils (pseudoeosinophils in rabbits) in the form of a fall in the first and a rise in the second; these changes are also maintained for a longer time. It is still possible, however, that the secondary leucocytosis and the shift in the formula are connected with reflex effects from the interoceptors of the blood vessels, but further and more detailed evidence is required on this problem.

There is no doubt that in addition to stimulating the hemopoietic tissue by the humoral route, chlortetracycline may also cause reflex changes in the composition of the leucocytes.

Differences in the action of chlortetracycline alone and with novocain may also serve, in particular, to explain clinical observations during the use of chlortetracycline in combination with ecmolin and novocain, as suggested by Z. V. Ermol'eva and her co-workers [9].

SUMMARY

Leukocytosis appears in rabbits after intramuscular administration of chlortetracycline in the dose of 25 mg/kg of body weight. It reaches its maximum in 2-4 hours and decreases or disappears completely in 5-10 hours. Then a second rise of the quantity of leukocytes occurs which reaches its maximum in 24-48 hours following the administration of chlortetracycline. The ratio of the lymphocytes and the segmented neutrophiles (pseudoeosinophils) is altered simultaneously with leukocytosis, with a decrease of the number of the lymphocytes and increase of pseudoeosinophils.

The first rise in the number of leukocytes depends on the reflex action of chlortetracycline on the blood system due to the excitation of the receptor apparatus of the muscle tissue. The second rise of leukocytes and the shift of the blood formula apparently depends upon the humoral action of the absorbed chlortetracycline on the hemopoietic organs.

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CULTIVATION OF TOXOPLASMA GUNDI IN TISSUE CULTURES

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(Received April 1, 1959. Presented by Active Member of the AMN SSSR N. N. Zhukov-Verezhnikov)

The method of tissue culture has been applied only comparatively recently to the study of the pathogenic protozoa and of the diseases which they cause. However as we know this method is widely used at the present time for all manner of work on different viruses, pathogenic to man and animals.

It is only in recent times that the method of tissue culture has begun to find application in the study of toxoplasmosis: the suitability of tissue cultures for the diagnosis of toxoplasmosis [5]; the quest for effective drugs for the treatment of toxoplasmosis; investigation of the action of toxoplasms on the cell [4]; the length of survival of toxoplasms in tissue culture [6] and so on. Despite the fact that still comparatively few investigations have been carried out in this field it is to be expected that this method will in future be used for a variety of purposes: maintenance of strains of toxoplasms; the study of the cytology, biology and metabolism of toxoplasms and so on; production of antigen for the complement fixation reaction and a skin test used in the diagnosis of toxoplasmosis; the diagnosis of toxoplasmosis by infection of tissue cultures with material from sick persons and animals; the study of some of the problems of pathogenesis and immunity, etc. in toxoplasmosis; research into effective chemotherapeutic drugs for use in toxoplasmosis.

EXPERIMENTAL METHOD

For this research we used cultures of various tissues from chick embryos.* Pieces of skin, muscle and heart were taken from 7-11 day old chick embryos. The tissues were grown on cover glasses in a nutrient medium of the following composition: Hanks' solution — 50%, chick embryonic extract — 10%, chick serum — 40%.

To 10 ml of this medium was added 1 drop of penicillin (2 ml of physiological saline to 200,000 units). A small droplet of chick plasma was placed on the cover glass and smeared over the surface. Pieces of chick embryonic tissue were carefully ground up in physiological saline and a few of the tiny fragments of tissue were then affixed to the cover glass prepared in the manner described above.

The coverglass and adherent tissue fragments was placed into a penicillin flask and into each flask was poured 1-1.5 ml of the nutrient medium composed as described above. The flasks were carefully closed with rubber stoppers and placed in an incubator (at a temperature of 37°C) at such an angle that the nutrient medium covered the glass with the tissue fragments. After 3-4 days this nutrient medium was poured away and replaced by fresh medium of the same composition. After 1-2 days a large zone of growth of fibroblasts was formed around the fragment. Cultures produced in this way were inoculated with toxoplasms from the peritoneal exudate of mice.

* The work was performed under the direction of Dr. of Biological Sciences D. N. Zasukhin.

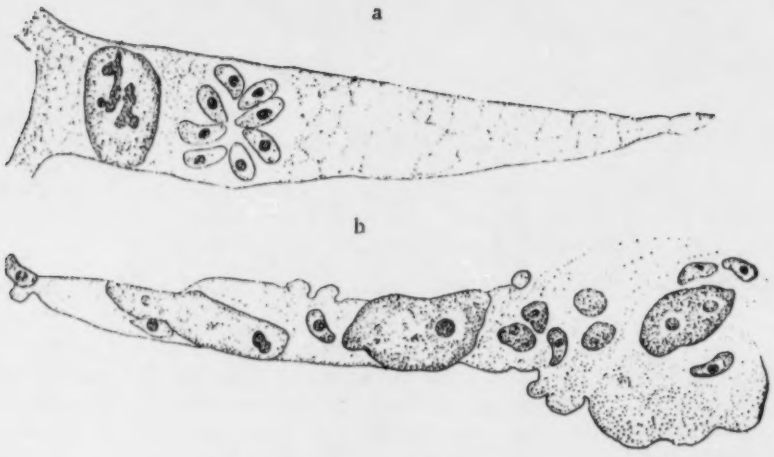

Fig. 1. A fibroblast in tissue culture 24 hours after infection. Toxoplasms arranged in rosette form in the cell; the protoplasm of the fibroblast is slightly vacuolated.
a) Destruction of a cell infected by a toxoplasm. Absorption of the protoplasm and karyorrhexis of the nucleus (b).

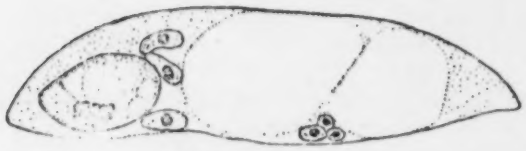

Fig. 2. A fibroblast infected with toxoplasm from a chick embryo. In the cell large vacuoles appear which gradually fill the whole cell and displace and distort the nucleus.

Before inoculation, the old nutrient medium was aspirated from the flasks. Next, 0.1 ml of exudate was added to the growing tissue and left for 1-1½ hours at room temperature. 1-1.5 ml of fresh nutrient medium was then added to the flask. In some flasks the medium was changed regularly every 3-4 days and in others it was not changed at all.

The material was fixed in alcohol and ether (50:50) 2, 3, 6, 23 and 29 hours and 1, 2, 3, 4, 5, 6 and 7 days after inoculation. The cultures were stained by the Romanowsky method (for 30 minutes at a temperature of 37°C). The film was taken through acetone mixtures and mounted in neutral Canada balsam. Control films from noninfected tissue cultures were made concurrently by the same method.

Besides experimental inoculation of healthy tissue we also carried out experiments in which tissue from chick embryos infected with toxoplasmosis was cultivated. For this purpose, 8-day chick embryos were opened up and fragments of muscle or heart explanted by the method described above. For cultivation a nutrient medium of the same composition was used. The material was fixed every 24 hours after inoculation.

EXPERIMENTAL RESULTS

Thirty-three experiments were performed, i. e. about 300 films were examined. Fixation was carried out, as has been pointed out, starting from 3 hours after inoculation of the tissue culture with toxoplasms.

After 2-3 hours we found toxoplasms of typical form in the cells. The infected cells were still very small, but individual cells sometimes were intensively infected. The parasites in the cells were solitary but in a few cases they reached 7 in number. The toxoplasms were arranged haphazardly in the protoplasm. No vacuoles were yet to be seen around them. No dividing parasites were observed at this period. In the infected cells we often saw mitoses of the nucleus and attenuation of the protoplasm of the cell leading, as we shall see later, to its absorption.

After 6 hours there were more toxoplasms in the growing fibroblasts. The average number of parasites per cell was 8. From time to time cells were encountered in which toxoplasms were to be found not only in the center of the cell but also in its numerous processes; in such cases they were 20-25 in number. The toxoplasms were arranged haphazardly in the cell protoplasm. Often accumulations of them deformed the nucleus; it was often markedly indented. The nucleus was often displaced towards the periphery of the cell.

On examination of a large number of films some areas presented a distinctive picture: toxoplasms (2-5 in number) lay directly against the nucleus. Whether this is due to penetration of the parasites into the nucleus or the result of superimposition of the parasites on the nucleus cannot yet be decided.

After 24 hours the toxoplasms occupied almost 1/3 of the cells. In many of the parasites a vesiculiform nucleus could be clearly distinguished. They were mainly arranged haphazardly in the cell but in some cells they formed rosettes (Fig. 1) or were arranged in a single row. The rosettes were usually made up of an even number of toxoplasms — 4, 6, 8 or 10. Parasites arranged singly or in pairs were often surrounded by vacuoles. Toxoplasms in a state of division were seen. At this time some cells were already undergoing destruction: all the protoplasm appeared "frothy" and its separate areas were becoming attenuated – the protoplasm was apparently being absorbed (Fig. 2).

After 48 hours the maximum number of toxoplasms was observed both inside and outside the cells. Before the maximum cytopathogenic effect had been achieved, the explantate contained a large number of free parasites. Those arranged in the form of rosettes were particularly numerous. The parasites were usually surrounded by vacuoles and the rosettes were always so surrounded. The bulk of the toxoplasms had undergone division: they were arranged in pairs unless division was not yet complete. Multinucleated fibroblasts (2-7 nuclei) were seen. Intensive cell destruction was observed and the further growth of the cells was retarded.

After 72 hours the majority of cells were now destroyed. The protoplasm was being absorbed and eventually only the bare nuclei were left. Often the nucleus itself was broken up into several segments. Those cells which had not yet disintegrated were highly vacuolated. Often a single, large vacuole occupied the center of the cell, and both protoplasm and nucleus (or nuclei) were displaced to the periphery.

There were many free toxoplasms in the center of the fragment, but in the zone of growth — now the zone of destruction — hardly any were present. Parasites which had escaped from the disintegrating cells found the conditions in the medium outside the cells unfavorable for life.

After 4-7 days the cells were completely destroyed and the toxoplasms had almost completely disappeared.

The observations described above were made on cultures in which the nutrient medium was not changed before and including the 4th day. We made a parallel study of control films in which none of the above changes were observed.

An insufficient number of experiments to cultivate tissue from infected chick embryos (see above) were carried out. At this stage we can only state that the cultivation of toxoplasms in this tissue is possible.

After 48 hours the greatest number of parasites was concentrated in the center of the fragment. However toxoplasms also appear in newly growing fibroblasts. It is true that there are very few infected cells. Inside the cells the toxoplasms are arranged either in a single row or, more often, haphazardly (Fig. 2).

On the 3rd day the toxoplasms develop most violently; their arrangement inside the cell is the same as on the 2nd day. The rosettes of parasites, which we saw in the experimental infection of normal chick tissue, are not observed here. Toxoplasms are also seen outside the cells. However on the 5th day after setting up the culture the number of toxoplasms is already decreasing and the cells are highly vacuolated. Free toxoplasms are encountered.

The methods described may be used in the future in the study of toxoplasms and of various problems in connection with the diseases caused by these parasites.

SUMMARY

It was demonstrated that toxoplasms may be successfully grown in the tissue cultures, causing a characteristic cytopathogenic effect. The parasites penetrate into the cells and by multiplying gradually fill them

completely. The nuclei are subjected to pyknosis and kariorrhexis; the protoplasm becomes vacuolized and is gradually resolved; the cell degenerates. Toxoplasms escape from the cell and penetrate intact, uninfected cells. The parasites perish when the tissue is completely destroyed.

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ONCOLOGY

PROLONGED HETEROGENIC TRANSPLANTATION OF MOUSE TUMORS IN RATS

COMMUNICATION II. A STUDY OF THE ANTIGENIC PROPERTIES OF CELLS OF EHRLICH'S ASCITIC MOUSE CARCINOMA DURING HETEROTRANSPLANTATION BY MEANS OF THE COMPLEMENT FIXATION REACTION

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The complement fixation reaction (CFR) is widely used in oncology for the immunological analysis of malignant tumors in man and animals. It has been shown by the CFR that tumors contain both species, organ, group and other antigens in common with normal tissues and also specific tumor antigens [2, 3, 8, 9, 10, 13, 16, 18]. The existence of the latter has also been proved by other immunological methods [5, 6].

The CFR has had only limited application to the study of the constancy of the antigenic structure of tumors during heterogenic transplantation and the investigations which have been done have yielded conflicting results [1, 7, 11, 14, 15, 17, 18]. In addition to its general biological interest, further study of this problem would allow the examination of the subject of vaccination against cancer from a new viewpoint [4].

The purpose of our work was to study, by means of the CFR, the antigenic properties of the cells of a mouse cancer undergoing prolonged heterotransplantation in rats.

EXPERIMENTAL METHOD

We employed a simple method of our own invention for transplanting an Ehrlich's ascitic mouse cancer into rats [12]. This method has definite advantages over those previously employed in the immunological analysis of tumors, since it enabled tumor cells to be obtained separately from ascitic fluid and, more important, it enabled them to be counted.

In the first place we obtained 3 types of immune serum in 15 rabbits (by Kapichnikov's method [6]): against ascitic mouse cancer cells (AMC), against mouse liver (ML) and against rat liver (RL). Only three sera (Nos. 1285, 1579 and 1082) were selected for the experiments whose titers were not less than 1:640 (i.e. they reacted + in this dilution with their own antigen).

The antigens used were saline extracts of fresh tissues (mouse cancer; heterotransplantates; mouse spleen, lung and liver; rat liver) and were prepared by the standard method. The tissue was thoroughly washed with physiological saline, ground in a mortar for 30 minutes; then to it was added physiological saline (0.85% NaCl) in a proportion of 10 ml to 1 g of tissue; the suspension was centrifuged for 15 minutes at 3000 rpm; the supernatant fluid was then aspirated with a Pasteur pipette and was used as antigen. As a preliminary measure the cancer cells were washed five times with physiological saline.

The CFR was performed in the usual manner at 37°C. As seen from Table 1, the serum used were reasonably specific.

TABLE 1

Typical Record of a CFR Experiment

Immune sera	Antigens	Dilutions of serum							Antigen Control (AC)
		1 : 20	1 : 40	1 : 80	1 : 160	1 : 320	1 : 640	1 : 1 280	
Against Ehrlich's ascitic mouse carcinoma (No. 1285)	Mouse cancer	++++	++++	++++	+++	++	++	+	h
	Heterotransplantate, 25th passage	++++	++++	++++	+++	++	++	+	h
	Mouse spleen	++++	+++	++	+	h	h	h	h
	Mouse lung	+++	++	+	h	h	h	h	h
	Mouse liver	+++	++	+	h	h	h	h	h
	Rat liver	++	+	h	h	h	h	h	h
Against mouse liver (No. 1579)	Mouse cancer	+++	++	+	h	h	h	h	Hemolytic system control (HSC)
	Heterotransplantate, 25th passage	+++	+	+	h	h	h	h	
	Mouse liver	++++	++++	++++	+++	++	+	h	++++
	Rat liver	+++	++	+	h	h	h	h	
Against rat liver (No. 1082)	Mouse cancer	+++	+	h	h	h	h	h	Complement control
	Heterotransplantate, 25th passage	+++	++	++	h	h	h	h	h
	Mouse liver	+++	++	+	h	h	h	h	
	Rat liver	++++	++++	++++	+++	+++	+	h	

Legend: CFR) complement fixation reaction; h) total hemolysis; + + +) complete prevention of hemolysis; + + +, + + and +) partial hemolysis of varying degree.

TABLE 2

Results of a Study of the Antigenic Properties of Cancer Cells from Heterotransplantates by Means of the CFR

Antigens	Immune rabbit sera																					
	Dilutions of sera																					
	Against Ehrlich's ascitic mouse cancer							against mouse liver						against rat liver								
	1 : 50	1 : 100	1 : 200	1 : 300	1 : 400	1 : 600		1 : 50	1 : 100	1 : 200	1:300	1:400	1:600	1 : 50	1 : 100	1 : 200	1:300	1:400	1:600			
1st passage MC	++++	++++	++++	++++	+++	++		++	+	h	r	r	r	++	r	r	r	r	r			
1st passage HTC	++++	++++	++++	++++	++	++		++	+	h	r	r	r	+++	+	r	r	r	r			
	1 : 20	1 : 40	1 : 80	1 : 160	1 : 320	1 : 640	1:1280	1 : 20	1 : 40	1 : 80	1:160	1:320	1:640	1 : 20	1 : 40	1 : 80	1:160	1:320	1:640			
25th passage MC	++++	++++	++++	+++	+++	++	+	+++	++	+	h	h	h	+++	+	h	h	h	h			
25th passage HTC	++++	++++	++++	+++	++	++	+	+++	+	+	h	h	h	+++	++	h++	h	h	h			
29th passage MC	++++	++++	++++	++++	++++	+++	++	+++	++	+	h	h	h	++	+	h	h	h	h			
29th passage HTC	++++	++++	++++	+++	+++	++	+	++	+	h	h	h	h	+++	++	+	h	h	h			
40th passage MC	++++	++++	++++	+++	+++	++	h	+++	++	h	h	h	h	++	+	h	h	h	h			
40th passage HTC	++++	++++	++++	+++	++	++	h	++	+	h	h	h	h	+++	++	+	h	h	h			
50th passage MC	++++	+++	++++	++++	+++	++	h	+++	++	h	h	h	h	++	h	h	h	h	h			
50th passage HTC	++++	++++	++++	+++	+++	++	h	+	r	h	h	h	h	++	+	h	h	h	h			
56th passage MC	++++	++++	++++	++++	+++	++	h	+++	++	h	h	h	h	++	+	h	h	h	h			
56th passage HTC	+++	+++	++	++	+	r	h	+	r	h	h	h	h	++	+	h	h	h	h			
61st passage MC	++++	++++	+++	+++	++	++	h	+++	++	+	h	h	h	+	h	h	h	h	h			
61st passage HTC	+++	++	+	+	r	r	h	+	r	h	h	h	h	++	h+	h	h	h	h			
70th passage MC	++++	+++	+++	++	++	+	h	++	+	h	h	h	h	+	r	h	h	h	h			
70th passage HTC	+++	++	++	+	r	r	h	r	r	h	h	h	h	++	+	h	h	h	h			

Note. MC) ordinary Ehrlich's ascitic mouse cancer; HTC) heterotransplantate; passage of mouse cancer through rats; remaining symbols as in Table 1.

Not less than 2-3 reactions were set up with each experimental antigen (heterotransplantate, passage) and in each case with 3 sera (AMC, ML, RL). Altogether 15 experiments were carried out.

Included in the investigation was material from 70 repeatedly transplanted heterogenic tumors corresponding to about one year in time. The heterotransplantates were studied selectively (after an average of 5-10 passages), and each time material from the original mouse tumor and also from mouse and rat liver was used as a control. The AMC serum was used to investigate the degree to which the original antigenic composition had been preserved in the cells of the cancer heterotransplantates; the ML serum to ascertain the degree of preservation of mouse antigens common to both mouse and liver and mouse cancer and the RL serum — the degree of acquisition of common antigens with rat liver by the cells of the cancer heterotransplantates.

All the CFR experiments included controls of antigens, complement, hemolytic system and sera.

EXPERIMENTAL RESULTS

The results of the investigation of the antigenic composition of the cells of the heterotransplanted tumor are summarized in Table 2 in which mouse cancer and its successive transplantates in rats are shown selectively.

As seen in Table 2, the mouse ascitic cancer antiserum (AMC) reacted with antigen of ordinary Ehrlich's ascitic mouse cancer in dilutions of 1:20 and 1:40 to + + + +, in dilutions of 1:80 and 1:160 — to + + + + and + + +, in dilutions of 1:320 and 1:640 — to + + + and + +, and in a dilution of 1:1280 — to + (and this only in the first CFR experiments when no fall in the titer of the antisera had yet been observed).

The AMC antiserum reacted equally intensively in the same dilution with the antigens of the 1st, 25th, 29th, 40th and 50th serial heterotransplantates. In the case of tumor antigens from the 56th, 61st and 70th passages in rats, however, the same antiserum reacted noticeably more feebly, i.e. in dilutions of 1:20 and 1:40 — to + + + and + +, in dilutions of 1:80 and 1:160 — to + + and +, and in dilution of 1:320 — to + (and this only with the 56th passage of the tumor).

It may also be seen in Table 2 that mouse liver antiserum (ML) reacted with antigen of the ordinary ascitic cancer in dilution of 1:20 to + + +, in dilution of 1:40 to + + and in dilution of 1:80 to +. ML antiserum reacted almost equally with antigens from the tumor after the 1st and 25th passages, rather less with antigens after the 29th and 40th passages and perceptibly more feebly with antigens after the 50th, 56th, 61st and 70th passages of the tumor.

The results given in Table 2 show that a positive reaction was obtained with antigens of the original tumor and RL antiserum in dilution of 1: 20 mainly to + + and +, in dilution of 1:40 to +, whereas with antigens from the tumor after the 1st, 25th, 29th, 40th, 50th, 56th, 61st and 70th passages the RL antiserum reacted in much higher titer: in dilution of 1:20 — to + + + and + +, in dilution of 1:40 — to + + and +, and sometimes (in the earlier experiments, i.e. up to the 50th generation) in dilution of 1:80 — to +.

It can be concluded from the results given in Table 2 that the antigenic composition of the cells of Ehrlich's ascitic mouse carcinoma is modified to some degree during transplantation in rats.

Of greatest interest is the "weakening" of the antigenic properties of the mouse cancer after the 40th-55th passage through rats, which corresponds in time to marked changes in the biological properties of the transplanted tumor [12].

There are, therefore, grounds for the belief that at this period the cancer cells lose some of their antigens which are common to the tumor and to mouse liver, i.e. some of the species antigens of the mouse.

With regard to the acquisition of antigens, characteristic of rat liver, by the transplanted tumor, we consider that in the first few passages this fact may be due to the simple adsorption of rat proteins on the cancer cells, although our results do not exclude the other possibility (inclusion of these proteins in the composition of the cancer cells). Further experiments are necessary to test these hypotheses.

The results obtained call for a much more profound immunological study, for they introduce fundamentally important questions of the mechanism of adaptation and variation of cancerous tissue.

SUMMARY

The antigenic properties of the cells of mice ascitic Ehrlich's carcinoma transplanted to rat's offsprings for a year was studied with the aid of the reaction of complement fixation. From the first transplantations the cells acquire low quantities of the rat's species-specific antigens, while in 40-55 reinoculations they lose a number of mice species-specific antigens. The loss of the mice antigens is associated with the decreased growth of the heterogenic strain of the tumour.

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THE ACTION OF A SPECIFIC IMMUNE SERUM ON THE DEVELOPMENT OF M-1 SARCOMA IN RATS

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A large volume of experimental research has shown that tumor antisera may modify the growth of a tumor and may injure tumor cells in experiments in vitro [3, 9, 10, 11, 13 and others]. These findings suggested that tumor antisera might also be effective in vivo and restrain the growth of tumors.

Observations showed that tumor antisera in some cases may depress cell division in malignant tumors [1, 8], and delay growth of certain tumors and the formation of metastases [5, 8, 9, 11, 14]. In other cases these sera have no effect whatsoever on the growth of tumors [16, 17].

In experimental research into the localization of tumor antibodies labeled with radioactive isotopes, it was shown [2, 13, 16, 18 and others] that these antibodies are present in both tumor tissue and the normal organs of the animal, for the immune sera used contained both tumor antibodies and antibodies common to normal and tumor tissues.

After P. N. Kosyakov and his co-workers [4] had devised a method of obtaining specific tumor antisera, we studied the action of a specific antiserum to Ehrlich's adenocarcinoma of mice on the development of an ascitic form of this tumor. The results of this study showed that the specific tumor antiserum has an effective action on the growth and development of the ascitic form of Ehrlich's adenocarcinoma of mice; large doses of the concentrated serum inhibit the growth of the tumor and, on the other hand, small doses stimulate it [6, 7].

The ascitic form of Ehrlich's adenocarcinoma of mice was a very convenient model for studying the action of this specific serum, for with experiments both in vitro and in vivo it was possible to create the best conditions for direct contact between specific antibodies and "pure" tumor cells.

It appeared to be of interest to study the action of a specific serum on the growth and development of another type of tumor — the M-1 sarcoma of rats. This was the purpose of the present experimental investigation.

EXPERIMENTAL METHOD

An M-1 sarcoma was cultivated in adult rats (140-180 g) by subcutaneous implantation (with a syringe) of a suspension of tumor tissue, chopped up finely with scissors in physiological saline (dose 0.2 cm^3). The tumor was implanted after 14-16 days.

Specific immune antisera to M-1 sarcoma and rat spleen were obtained by immunization of rabbits. The spleen antiserum had an antibody titer in the complement fixation reaction of 1:400. We used this as an antireticulocytotoxic serum and designated it ACS. The titer of specific antibodies in the adsorbed tumor antiserum was 1:160 to 1:80.

Experiments to study the action of the tumor antiserum on tumor tissue in vitro. Under the most sterile conditions possible the tumors in the rats were excised, freed from necrotic areas and cut up finely with ophthalmic scissors or a high-speed mincer. Samples of equal weight of the minced tumor tissue were placed in

TABLE 1

The Action of Various Dilutions of Specific Tumor Antiserum on Tumor Transplantates of M-1 Sarcoma in Rats (in vitro)

Group of rats	Treatment of tumor transplantate before inoculation	Experiments									
		1		2		3		4		5	
		number of rats included in experiment	average weight of tumor (g) on the 19th day	number of rats included in experiment	average weight of tumor (g) on the 20th day	number of rats included in experiment	average weight of tumor (g) on the 21st day	number of rats included in experiment	average weight of tumor (g) on the 14th day	number of rats included in experiment	average weight of tumor (g) on the 21st day
1 experimental	Tumor antiserum in a dilution of 1 : 10	30	2,1 (P=0,0278)	32	2,5 (P=0,0574)	26	11,1 (P=0,2713)	23	3,8 (P=0,0019)	28	0,48 (P=0,0124)
2 experimental	The same 1 : 60	29	5,7 (P=0,2301)	33	6,8 (P=0,4237)	26	12,2 (P=0,0799)	23	7,2 (P=0,000)	15	2,8
3 control	Physiological saline	31	4,1	31	5,2	25	8,0	23	1,7	28	2,1

flasks. To one flask was added specific tumor antiserum in a dilution of 1:10, to the second tumor antiserum in a dilution of 1:60, and to the third physiological saline (3-4 cm^3 of serum or physiological saline to 1 g of tumor tissue). The suspensions obtained were kept for 2 hours in the incubator and then they were injected subcutaneously into rats in a dose of 0.2 cm^3.

On the 14th - 21st day after inoculation the rats were killed and the tumors weighed. The mean weight of the tumor was determined for all the rats of each group. The values obtained for the mean weight of the tumor were treated statistically. The results were regarded as significant if the value of P was less than 0.05.

Experiments to study the action of the serum in vivo. Tumor antiserum was injected subcutaneously or intravenously a few hours before or from the first day after inoculation of the tumor in a dose of 0.1 to 2 cm^3, diluted 1:10, 1:20 or 1:40, from 4 to 10 injections being given either daily or at intervals of two days. In some experiments injection of tumor antiserum was combined with small doses of ACS (1 cm^3 of a 1:20,000 dilution) or with guinea pig serum.

Control rats were injected with physiological saline or normal rabbit serum, or were left quite untreated.

On the 25th - 44th day after inoculation of the tumor the rats were killed.

The tumors were weighed and the mean weight in grams calculated. In some experiments in the course of development of the tumor its length and breadth were measured through the skin and the mean area of the tumor in cm^2 was calculated.

EXPERIMENTAL RESULTS

Effect of the serum on M-1 sarcoma in vitro. In Table 1 are shown the results of experiments in which the tumor tissue was treated before inoculation with tumor antiserum (1:10 or 1:60) and with physiological saline (control).

Comparison of the mean weight of the tumor in different groups of animals shows that tumor antiserum in a dilution of 1:10 has a depressing action on the viability of tumor tissue. The average weight of the tumor in the rats of this group in experiments Nos. 1, 2 and 5 was considerably less than the average weight of the tumors in the animals of the control groups. Depression of the growth of the tumor by the action of the serum was statistically significant

TABLE 2

The Action of Specific Tumor Antiserum on the Development of M-1 Sarcoma in Rats in Experiments in Vivo

Experiment	Group of rats	Treatment of rats	No. of rats incl. in expt.	No. of rats with tumors on day killed	average weight of tumor, g
1	1	10 subcutaneous injections of tumor antiserum each of 1 cm^3 (1:10) and of 1 cm^3 of ACS (1:20,000)	8	6	18.1
	2	Without treatment	13	8	7.45
2	1	10 subcultaneous injections of tumor antiserum each of 1 cm^3 (1:10) and of 1 cm^3 of ACS (1:20,000)	12	4	1.37
	2	10 subcutaneous injections each of 1 cm^3 of ACS (1:20,000)	10	0	0
	3	Without treatment	10	0	0
3	1	10 subcutaneous injections of tumor antiserum each of 1 cm^3 (1:10) and of 1 cm^3 of ACS (1:20,000)	29	23	7.2
	2	10 subcutaneous injections each of 1 cm^3 of ACS (1:20,000)	27	18	4.2
	3	4 subcutaneous injections of 1 cm^3 of normal rabbit serum (1:100)	15	11	9.1
	4	Without treatment	13	7	15.5
5	1	5 intravenous injections of 2 cm^3 of tumor antiserum (1:10)	20*	6	0.41
	2	5 intravenous injections of 0,1 cm^3 of tumor antiserum (1:10)	19	12	0.64
	3	5 intravenous injections of 2 cm^3 of normal rabbit serum (1:10)	20	12	0.99
	4	5 intravenous injections of 0,1 cm^3 of normal rabbit serum (1:10)	13	8	1.0
	5	Without treatment	22	12	0.55
6	1	5 injections of 2 cm^3 of tumor antiserum (1:10) subcutaneously and intravenously	30**	12	20.9
	2	5 injections of 2 cm^3 of tumor antiserum	31	26	17.4
	3	Without treatment	28	25	16.1

* Four rats in this group died from injections of serum.

** Eighteen rats in this group died after 3 injections of serum.

in these experiments [in experiment No. 1 — P = 0.0278, experiment No. 2 — P = 0.0574 (on the borderline of significance) and experiment No. 5 — P = 0.0124].

In the groups of rats which were inoculated with tumor tissue treated with tumor antiserum diluted 1:60 growth of the tumor was stronger than in the controls.

In all the experiments the average weight of the tumor in the rats of these groups exceeded the average weight of the tumor in the rats of the control groups, although only in experiment No. 4 was this stimulation statistically significant (P = 0.000).

In two experiments (Nos. 3 and 4) stimulation of the growth of the M-1 sarcoma was also observed in the groups of rats inoculated with tumor tissue that had been treated with tumor antiserum diluted 1:10.

TABLE 3

Stimulation of Growth of an M-1 Sarcoma in Rats by Specific Tumor Antiserum

Group of rats	Treatment of rats	No. of rats incl. in expt.	Average area of tumor (cm^2) on the 15 day	Average area of tumor (cm^2) on the 20 day	Average weight of tumor (g) on the 21 day
1 experimental	10 injections of 1 cm^3 of specific serum (1:10)	10	4.8 (P=0.041)	10.4 (P=0.089)	16.8 P=0.151)
2 experimental	10 injections of 0.5 cm^2 of specific serum (1:10) and 0.5 cm^2 of guinea pig serum	10	4.3 (P=0.041)	9.2	16.8
3 control	Without treatment	10	2.0	5.3	8.1

Stimulation of the growth of the tumor transplantates by the action of the serum in dilution of 1:10 may evidently be due to the different degree of mincing of the tumor tissue in the various experiments. Poorly minced tissue fragments elicit a weaker action from the specific antibodies, i.e. in these experiments a dilution of 1:10 was the low, stimulating dose of the serum.

The experiments carried out show that concentrated specific tumor antiserum lowers the viability of tumor cells; a smaller concentration of serum on the other hand may stimulate the viability of the tumor tissue and encourage growth of the tumor.

Experiments with M-1 sarcoma in vivo. In order to study the action of the specific serum on the growth of M 1 sarcoma we undertook a series of experiments, the results of which are shown in Table 2. In experiments Nos. 1 and 2 the rats of the experimental groups received daily subcutaneous injections of 1 cm^3 of specific serum diluted 1:10 and of 1 cm^3 of ACS diluted 1:20,000 for 10 days, starting on the 1st day after inoculation of the tumor.

Comparison of the mean weight of the tumor in rats treated with the sera and in the controls showed that injection of the sera is accompanied not by inhibition but, on the contrary, by some degree of stimulation of growth of the tumor. However, the differences between the average weight of the tumors in the treated and control rats were not statistically significant (experiment No. 1 — P = 0.0888; experiment No. 2 — P = 0.105). In experiment No. 3 (see Table 2) some retardation of growth of the tumor was observed after injection of specific tumor antiserum and a small dose of ACS, but the differences between the average weights of the tumors of the experimental and control groups were not statistically significant.

In view of the fact that the dose of specific serum injected (1 cm^3 daily for 10 days) did not materially affect the growth of the M-1 sarcoma, in subsequent experiments we increased the dose of each injection of serum to 2 cm^3.

These experiments (Nos. 5 and 6) showed that the injection of large doses of specific serum had no action whatsoever on the development of the tumor. Intravenous injections of large doses of the foreign serum led to death of the animals.

In experiment No. 7 (the results of which are given in Table 3) the serum was injected intravenously the first time, 3 hours before inoculation of the tumor. Subsequent injections were given subcutaneously, starting on the first day after inoculation of the tumor, with intervals of 1-2 days (dose 1 cm^3). In this experiment stimulation of growth of the tumor by the injections of serum was observed.

Measurement of the mean area of the tumor on the 15th day after inoculation showed that its value in the control group was 2 cm^2, whereas in groups Nos. 1 and 2, which received tumor antiserum, the mean area of the tumor was 4.8 and 4.3 cm^2 (the difference between these groups and the control is statistically significant — P = 0.041).

On the 20th day the average area of the tumor in the treated rats also exceeded the average area of the tumor in the control animals but the difference was not significant (P = 0.089). On the 21st day the rats were killed. The average weight of the tumor in groups Nos. 1 and 2 was 16.8 g and in the control group — 8.1 g (difference not statistically significant: P = 0.151).

Comparison of the experimental research which we carried out on the effect of specific sera on the growth and development of the ascitic form of Ehrlich's adenocarcinoma of mice and of M-1 sarcoma in rats shows that the cells of Ehrlich's adenocarcinoma were more amenable to the action of specific antibodies in experiments both in vitro and in vivo. Both in vitro and in the peritoneal cavity of mice, the latter had a direct destructive action on "pure" tumor cells of Ehrlich's adenocarcinoma.

When studying the action of the specific serum on M-1 sarcoma transplantates we did not possess "pure" tumor cells. The serum acted on the whole fragment of tumor tissue, exerting its damaging influence on the superficial layers only. Evidently for this reason the depressing influence of the tumor antiserum was shown less clearly.

Tumors developing in animals were even less amenable to the action of the specific antibodies. The experiments showed that injection of rats subcutaneously or intravenously with up to 10 cm^3 of specific serum had no material influence on the development of an M1 sarcoma. In no experiment were we able to obs[illegible]e diminished growth of the tumor among the group of rats in which tumor antiserum was given alone or in conjunction with a small dose of ACS and normal guinea pig serum.

Wissler [18] explained the absence of inhibiting action of tumor antiserum on the growth of the Flexner-Jobling sarcoma and the accumulation of a higher concentration of tumor antibodies in normal organs than in tumors by the impermeability of the capillary walls to antibodies.

The experimental investigations which we carried out with the two different tumors may assist in the explanation of the failure of many research workers to influence the actively growing tumors of man and experimental animals by intravenous and subcutaneous injections of antisera.

Experiments with Ehrlich's adenocarcinoma of mice and M-1 sarcoma of rats have shown that an antiserum is only effective when conditions are present for the direct contact of the antibodies with the cells.

In some experiments in vitro and vivo stimulation of growth of an M-1 sarcoma by the use of tumor antiserum was observed. These findings of the stimulating action of sera which we obtained in the case of two different tumors suggest that in the clinical trial of tumor antisera it is essential to select the doses of the serum very carefully and to pay attention to their titer, since in place of depression of growth of the tumor stimulation may result.

SUMMARY

The author studied the effect of specific antitumor serum on the transplants of M-1 rat's sarcoma in experiments in vivo and in vitro. It was demonstrated that the treatment of these transplants with the specific serum (1:10 solution) decreases their vital capacity. On the contrary, a more diluted solution of this specific serum may have a stimulating effect on the M-1 sarcoma transplants. Administration of up to 10 cc of the specific antitumor serum (1:10 solution) had no significant effect on the development of M-1 sarcoma. No inhibition of the tumor growth could be noted in any of the experiments. On the contrary, in certain experiments administration of the serum was associated with the stimulation of the tumor growth.

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SOME PECULIARITIES OF METASTASIS FORMATION BY EXPERIMENTAL TUMORS

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The study of the process of metastasis formation by malignant tumors occupies an important place in experimental oncology. Despite the extensive literature on this subject, opinions are still divided on the causes of metastasis formation by malignant tumors. Of great interest to research workers in this field is I. P. Pavlov's view of the growth of malignant tumors [5]. He believed that stimulation arising from the tumor focus, acting through a nerve reflex, can affect the nutrition of the tissues surrounding the tumor, thereby creating favorable conditions for growth of the tumor cells. I. P. Pavlov's views on the importance of trophic reflexes for the growth of tumors provided a basis for the explanation of metastasis formation and they were accordingly developed further in research by a number of workers [3, 4, 7, 8 and others].

S. I. Lebedinskaya and A. A. Solov'ev [3, 4] showed that the application of direct trauma to peripheral nerves may lead to enhanced metastasis formation and may alter the distribution of the metastases. In view of their findings, these workers consider that nervous reflex mechanisms lie at the basis of the process of metastasis formation, and determine not only the appearance of metastases but also their location. Some workers [2, 10-13] have also pointed out a definite role in metastasis formation is played by the biological peculiarities of malignant tumors. All these investigations show that the formation of metastases by malignant tumors is a complex process which depends on various factors of the external and internal environments.

The object of the present work was to study the relationship of the development of metastases to the individual peculiarities of a number of experimental tumors and to the site of introduction of the primary tumor material. We attempted to discover the strain of tumor which would be the most suitable model for the experimental study of the laws of metastasis formation.

EXPERIMENTAL METHOD

Our investigations were carried out on Ehrlich's adenocarcinoma of mice and on various tumors of rats (M-1 sarcoma and Yoshida's sarcoma, produced in rats by carcinogenic substances; Guérin's carcinoma, deriving its origin from spontaneous carcinoma of the uterus; spontaneous tumor of the mammary gland — fibroadenoma). Metastases in the experimental animals were examined macroscopically at autopsy and, in doubtful cases, microscopically.

Ehrlich's adenocarcinoma was inoculated in mice by implantation of ascitic cells in a dose of 0.2-0.5 ml of undiluted ascitic fluid in the peritoneal cavity or under the skin of the left or right flank. The rats were inoculated subcutaneously, intraperitoneally and intratesticularly with tumor tissues. Yoshida sarcoma was injected in the form of ascitic cells and a suspension of tumor fragments in physiological saline. The remaining rat tumors were inoculated by a suspension of tumor fragments only. The dose of material inoculated was the same in all cases in the experiment, namely 1 ml of a 10% suspension of tumor fragments. The experimental animals were of both sexes and were aged from 3 to 8 months.

EXPERIMENTAL RESULTS

Ehrlich's mouse adenocarcinoma. 145 mice were inoculated subcutaneously with Ehrlich's adenocarcinoma. Of these mice, 60 died from tumors between the 10th and 30th days. No metastases from Ehrlich's adenocarcinoma were found in any of the mice dying at this time. Of the 48 experimental animals dying from tumors between the 31st and 35th days, one mouse (2.08%) had metasteses in the lymphatic glands. The number of mice with metastases rose as the experimental animals died at later periods. For example, among animals dying between the 36th and 40th days metastases were observed in 9 out of 21 (44.4%), and of the 16 mice dying after the 40th day, metastases were found in 10 (62.5%). Hence, after subcutaneous inoculation of Ehrlich's adenocarcinoma metastases developed in 20 of 145 mice, or in 13.7% of cases. Metastases were found in the omentum, peritoneum, kidneys and lymphatic glands.

The highest percentage of metastases of Ehrlich's adenocarcinoma was found when the tumor was inoculated intraperitoneally. Of the 120 mice in this experiment, 115 showed the presence of metastases (95.8%). In this case the metastases were located in the omentum, mesentery, sex organs, kidneys, liver, spleen and other organs.

The experimental results show that Ehrlich's adenocarcinoma of mice metastasizes feebly after subcutaneous inoculation and much more intensively when inoculated intraperitoneally. The highest percentage of metastases from Ehrlich's adenocarcinoma after subcutaneous inoculation was observed in our experiment between the 36th and 45th days, and not at early periods as described by S. Ya. Klenitskii [1].

M-1 sarcoma of rats. In this experiment 180 rats were inoculated subcutaneously and the inoculation was successful in 127 animals. In no cases, whether the rats died at an early or late period from the tumor, were metastases found.

When M-1 sarcoma was inoculated into the testicle, metastases developed in the peritoneal cavity of 87 of the 150 rats, or in 58% of cases. The metastases were situated in the omentum, mesentery or peritoneum, on the surface of the diaphragm and in muscle tissue. Thus in contrast to Ehrlich's adenocarcinoma of mice, the M-1 sarcoma of rats does not form metastases when inoculated subcutaneously. The development of metastases from this tumor is observed in experimental animals only when it is inoculated intratesticularly and intraperitoneally.

Yoshida's sarcoma of rats. This tumor was injected subcutaneously into 70 rats. Tumors developed in 62 animals (88.5%). At postmortem examination of rats dying from tumors, no metastases were seen in any animal. After intratesticular inoculation of rats with this tumor, metastases developed in the peritoneal cavity of 31 of the 40 animals. Metastasis formation from Yoshida's sarcoma has a much more intensive course when the ascitic cells of this tumor are injected intratesticularly than when a suspension of tumor fragments in physiological saline is used for the inoculation.

This is evidently due to the fact that ascitic cells of Yoshida's sarcoma are more malignant than the cells of the solid form of this tumor.

A high percentage of metastases is observed after intraperitoneal inoculation of Yoshida's sarcoma. In this experiment metastases developed in 45 of the 50 rats, or in 90% of cases. When Yoshida's sarcoma was inoculated intratesticularly and intraperitoneally, the metastases were situated mainly in the omentum, mesentery and muscle tissue, and on the surface of the diaphragm, i.e. just as in the case of metastasis formation from the M-1 sarcoma.

Guérin's sarcoma of rats. Ninety-five rats were inoculated subcutaneously with Guérin's carcinoma; 2 rats died on the 18th day without metastases. Thirty rats died from tumors between the 21st and 30th days. At postmortem examination it was found that only 2 rats had metastases in the lymphatic glands (cervical and inguinal). Of the 50 rats dying from tumors between the 31st and 35th days, metastases developed in 15 or in 30% of cases. Of 8 rats dying between the 36th and 40th days, 7 showed the presence of metastases (87.5%). Finally, all 5 rats dying at later periods were affected with metastases (100%).

Metastases from Guérin's carcinoma following subcutaneous inoculation were situated at later stages not only in the lymphatic glands but also in the peritoneal cavity, thyroid gland, adrenals, omentum, muscle tissue and mesentery. However the favorite site for the formation of metastases from Guérin's carcinoma after subcutaneous inoculation is the lymphatic glands. After intratesticular inoculation of rats with Guérin's carcinoma, the course of development of the tumor is somewhat different from that of the M-1 sarcoma or Yoshida's sarcoma

Development of a fibroadenoma of the mammary gland after its autotransplantation into the peritoneal cavity of an experimental rat. On the 124th day of the experiment massive tumor nodes are seen in the omentum, on the surface of the diaphragam and in the mesentery.

inoculated into the same organ. In our experiment the development of Guérin's carcinoma in the testicle was accompanied by relatively feeble matastasis formation, which in some rats was confined to the tissues close to the testicle. Of the 50 rats used in this experiment, metastases from Guérin's carcinoma developed in the peritoneum of 17 animals, or 34% of cases. The first metastases were formed in the omentum, mesentery and peritoneum. In some rats no metastases were observed in the peritoneal cavity during development of a Guérin's carcinoma in the testicle. In this case the tumors grew as a continuous conglomerate mass, quite separable from the adjacent tissues. This fact is all the more interesting because the testicle, according to the reports of many workers, is the organ from which metastases are formed by tumors of even a low degree of malignancy.

When inoculated intrapertoneally, Guérin's carcinoma metastasized in 19 of the 30 rats, or in 63.3% of cases. In some rats the development of Guérin's carcinoma in the peritoneal cavity was accompanied by ascites formation. The ascitic fluid contained blood corpuscles and the characteristic malignant cells of the ascitic form of the tumor. Subcutaneous inoculation of rats with these cells caused the development of typical carcinomas with a relatively short latent period of 2-3 days, followed by death of the rats on the 20th-25th day.

Thus in relation to its ability to form metastases, Guérin's carcinoma of rats differs essentially from the M 1 sarcoma and Yoshida's sarcoma of rats. After subcutaneous inoculation, Guérin's carcinoma metastasizes in 30.5% of cases, but the sarcomas do not form metastases in this case. Furthermore Guérin's carcinoma metastasizes directly in the internal organs (lymphatic glands, thyroid gland); according to our findings, these organs are not affected by metastases from M-1 and Yoshida's sarcomas.

Spontaneous mammary gland tumor (fibroadenoma) of rats. Fibroadenoma of the mammary gland is known to be a malignant tumor and consequently not to form metastases. The animals die from general exhaustion because of the intensive growth of this tumor.

In our experiment between 1953 and 1958 we observed 6 cases of fibroadenoma of the mammary gland in rats, and in no case were metastases formed from this tumor. Homotransplantation of a fibroadenoma intratesticularly in 9 rats and intraperitoneally in 26 rats was unsuccessful in all cases, in spite of the fact that the rats inoculated were genetically similar to the donors. In our experiments we therefore employed the generally known method of autotransplantation, described in detail by N. N. Petrov [6]. Autotransplantation of the fibroadenoma was carried out subcutaneously and intraperitoneally. For intraperitoneal inoculation 6 rats affected by tumors were used; in 4 of these animals massive tumor nodes weighing from 90 to 120 g developed in the peritoneal cavity after 3½ to 4 months. Tumor nodes were located in the omentum and mesentery and on the surface of the diaphragm.

This fact suggests that a fibroadenoma of the mammary gland may grow and develop not only at the site of its original appearance but in other parts of the body also. Evidently the peritoneal cavity is the most favorable environment for the growth of benign as well as malignant tumors.

It may be concluded from the results obtained that Güerin's carcinoma of rats is the most suitable for studying the process of metastasis formation by malignant tumors, for it forms metastases when inoculated subcutaneously, intratesticularly and intraperitoneally. Ehrlich's adenocarcinoma of mice metastasizes less intensively than Guérin's carcinoma when inoculated subcutaneously, and so is less suitable for studying this process. The M-1 sarcoma and Yoshida's sarcoma of rats were least suitable of all for the experimental study of the process of metastasis formation.

The metastasization of malignant tumors is a complex process in which, besides other factors, a large part is played by the situation of the primary tumor and its biological peculiarities. A benign tumor, such as a fibroadenoma of the mammary gland, may also grow when cells from it are introduced into the peritoneal cavity of the animal affected by the primary tumor. Under these circumstances, however, the internal organs (liver, kidneys, spleen,lungs,etc) are not affected by metastases.

SUMMARY

Metastasis of transplantable malignant tumors is a complicated process in which the localization of the primary tumor and its biological peculiarities together with other factors play an important role. Guérin rat's carcinoma, which metastasizes into internal organs both in subcutaneous inoculation and in its graft into the testicle or into the abdominal cavity, was found to be the most suitable for the study of the laws of metastasis of malignant tumors. Rat's M-1 sarcoma and Yoshida's sarcoma arethe least suitable for the study of this process.

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THE EFFECT OF HYPOTHERMIA ON METASTASIS OF BROWN-PEARCE TUMORS

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Cooling of the body as a whole was first used clinically as a method of influencing the growth of malignant tumors by Fay, Henny and Smith in 1938-1944 [17, 18, 20]. These workers made observations on 175 patients suffering from advanced forms of malignant disease, and came to the conclusion that general cooling of the body to a temperature of 27-29°C causes retardation of growth of the tumor and delays the development of metastases. Other workers came to similar conclusions: McCravey [19], Vaughan [22], Talbot [21] and others.

Nevertheless this method has not been widely used in oncological practice, since cooling has itself been found to be dangerous to the patient's life. The danger of cooling to higher animals is that when the temperature falls, a defensive reaction develops in the body which aims to maintain the body temperature at a constant

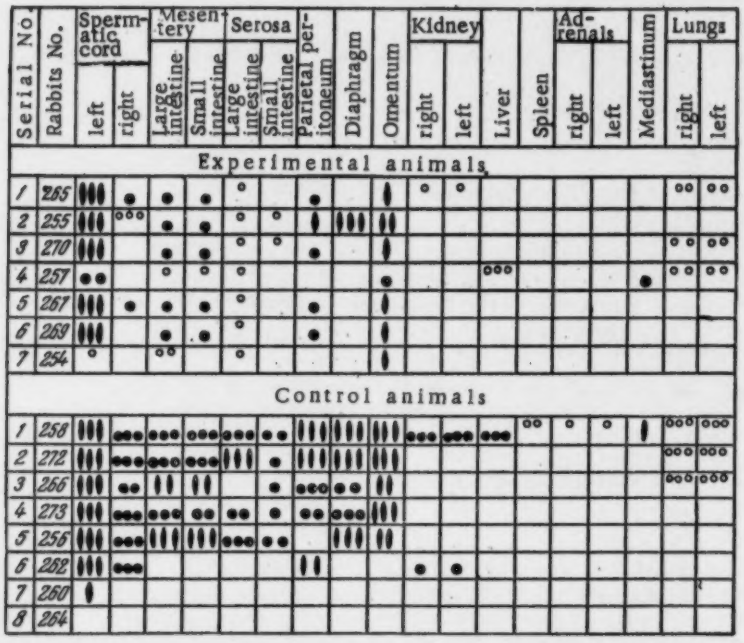

Fig. 1. Metastasis of a Brown-Pearce tumor in rabbits after six applications of hypothermia (1st series of experiments).

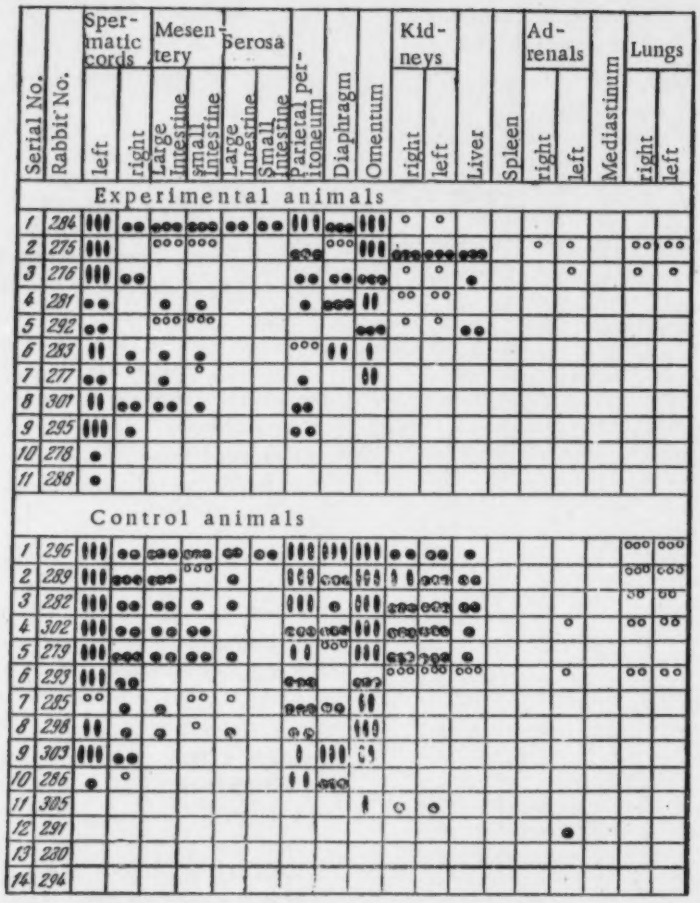

Fig. 2. Metastasis of a Brown-Pearce Tumor in rabbits after six applications of hypothermia (2nd series of experiments).

level which is required for the normal vital activity of the more highly organized animals. The development of this defensive reaction is associated with marked stimulation of metabolism, excitation of the sympathetic nervous system, increased secretion of adrenalin and an increase in the oxygen demand of the body. All this leads eventually to exhaustion of the body's resources of energy and to the development of profound limiting inhibition in the central nervous system [1, 4, 7 and others].

In recent years the method of hypothermia has been applied on a wide scale in a number of clinics, mainly surgical during traumatizing operations. In order to produce a state of hypothermia the majority of workers use a combination of physical cooling, anesthesia and drugs which block the autonomic nervous system. It has been found that such a combination reduces the danger of the cooling itself and is therefore the best method of producing hypothermia [3, 9 and others].

A malignant tumor, as a part of the body, is known to be dependent on the state of the body as a whole and on the state of its metabolic processes. Any change in this state affects the growth and development of the tumor [2, 8, 10, 12, 13 and others]. It may therefore be postulated that a change in the processes of metabolism in the body due to the action of low temperatures will also modify the development of the tumor.

The experimental research on this problem [5, 6, 14-16 and others] is unfortunately very scanty and very conflicting.

Comparative Incidence of Metastases in Various Organs of Cooled and Control Animals (1st and 2nd series of experiments)

Organs	Incidents of involvement of individual organs (in %)	
	in the experiment	in the control
Spermatic cords:		
left	100	77.2
right	50.0	72.7
Mesentery		
large intestine	77.7	54.5
small intestine	72.2	54.5
Serous membrane:		
large intestine	44.4	45.4
small intestine	16.6	27.2
Parietal peritoneum	72.2	68.1
Diaphragm	33.3	58.0
Omentum	77.7	68.1
Kidneys:		
right	33.3	40.9
left	33.3	40.9
Liver	22.2	31.9
Spleen	—	22.2
Adrenals:		
right	5.5	4.5
left	16.6	18.1
Lungs:		
right	26.0	36.0
left	26.0	36.0

In view of the foregoing we carried out investigations of the process of metastasis formation in association with the metabolic changes of hypothermia.

EXPERIMENTAL METHOD

Hypothermia was produced by a combination of physical cooling, anesthesis and injection of neuroplegic drugs. Experiments were carried out on 40 rabbits (grey chinchilla breed, weight 2100-2850 g; 18 experimental and 22 control animals). The experiments were performed in 2 series. Under aseptic conditions a Brown-Pearce tumor was implanted in the left testicle of all the rabbits, in the form of 0.6 ml of a 20% suspension of the tumor in physiological saline. For the inoculation we used metastatic nodes from the peritoneal cavity of rabbits killed on the 21st day after inoculation of this strain of tumor.

Hypothermia was produced as follows. A 25% solution of urethane was injected into the vein of the left ear (dose 0.8 g per 1 kg body weight). Two minutes after sleep developed an intramuscular injection of 0.1% chlorpromazine solution was given (1 mg per 1 kg body weight), with the addition of 0.1 ml of a 0.1% solution of atropine. The animal was then bound to a frame and ice-bags applied. After 20 minutes a further injection of half the previous dose of chlorpromazine was given. The temperature was measured by a mercury thermometer in the rectum every 30 minutes. As a rule the animal remained in a state of sleep throughout the experiment. From 1½ to 4 hours after the beginning of cooling the body temperature fell to 28-27°C.

Each rabbit of the experimental group was cooled 6 times, on the 3rd, 6th, 9th, 12th, 15th and 18th day after inoculation of the tumor. All the animals of the control and experimental groups were killed 21 days after inoculation. Throughout the experiment the experimental and control animals were kept on identical diets.

In our study of the process of metastasis formation we compared the incidence and degree of metastasis formation in individual organs in the cooled and control animals. We assessed the degree of metastasis formation by the number and size of the metastases in the organs. The size of the metastases is shown on the records as follows: metastatic nodes measuring up to 0.2 cm in diameter are indicated by a ring, those measuring from 0.2 to 0.5 cm in diameter — by a black circle, and those over 0.5 cm in diameter — by an oval. The number of metastases is indicated by the number of rings: up to 10 metastases in the organ — by one ring (solitary metastases) from 10 to 30 metastases — 2 rings (moderate number of metastes) and over 30 metastases in the organ — by 3 rings of the appropriate pattern (multiple metastases).

EXPERIMENTAL RESULTS

The experimental findings show that metastasis formation differed considerably in the experimental and control animals: in the experimental group of rabbits the degree of metastasis formation was less than in the controls. In the 1st series of experimental animals, for instance, large metastases were present in the various organs 13 times, and in the control group — 24 times. Metastases of moderate size were present in various organs of the experimental group of animals 19 times, and in the control group 29 times. Small metastases were observed in the organs of 23 experimental animals and of 9 controls.

The same relationship was noted when the number of metastases in the organs of the animals of the experimental and control groups was compared. In the experimental group, for instance, multiple metastases were present in the various organs of 8 animals, and in the control group, of 40 animals. A moderate number of metastases were observed in the organs of 9 experimental animals and 13 controls. Solitary metastases were seen in the organs of 38 experimental and 9 control animals (Fig. 1).

Precisely the same results were obtained in the 2nd series of experiments, in which the same method was used (Fig. 2).

The experimental results thus showed that in the rabbits undergoing hypothermia, small and solitary metastases were preponderant, whereas in the control animals the majority of the metastases were multiple and large in size.

The comparative incidence of metastases in the various organs of the cooled and control animals is shown in the Table (1st and 2nd series of experiments).

As seen from the table, no clear difference in the incident of involvement of individual organs of the experimental and control groups could be observed. However it may be pointed out that in the control animals metastases were more often observed in organs affected by hematogenous spread (liver, kidneys, lungs and so on), whereas in the experimental animals these organs were less commonly involved.

The results described are in agreement with earlier observations on the effect of one, two and three applications of hypothermia on the formation of metastases and the length of survival of experimental animals. In these investigations there was a fall in the intensity of metastasis formation and an increase in the average length of survival in the animals subjected to the hypothermia. It may be assumed from these findings that hypothermia delays the growth of metastases.

SUMMARY

The effect of hypothermia induction (6 times) on the metastasis of Brown-Pearce tumor was studied. Hypothermia was attained by employment of physical cooling combined with the administration of aminazine, urethane and atropine. The degree of metastasis was compared in experimental and control rabbits in 21 days after the transplantation. It appeared that the induction of hypothermia decreases the degree of metastasis.

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SOME PROPERTIES OF HUMAN "LEUKEMIC FACTOR" WHEN CULTIVATED ON THE EMBRYONIC MEMBRANES OF CHICKS

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Our previous investigations [1] showed that a noncellular factor (human "leukemic factor") is present in the tissues of patients with leukemia, and this may be cultivated on the membranes of developing chick embryos. It was also shown that after cultivation on the chorionallantoic membrane this factor possesses specific antigenic activity.

It appeared necessary (from the theoretical and, possibly, also from the practical point of view) to study some other important properties of the cultivated agent.

The present paper describes the results of experiments to investigate the effect of formalin, high temperatures, freezing and drying on the activity of "leukemia factor" after cultivation on the embryonic membranes of chicks. We also studied the electron microscopic picture of the cultivated factor. We tried, further, to discover whether it was possible for the "leukemia factor" to be adsorbed on red cells. The answers to these questions might be of considerable importance to the elucidation of the nature of human "leukemia factor."

EXPERIMENTAL METHOD

Blood and brain tissue filtrate from patients with leukemia were cultivated on the chorionallantoic membrane of fertilized chick embryos. The allantoic fluid of the inoculated embryos was subjected to various treatments (formalinization, heat, freezing and drying, and adsorption on red cells).

At the 6th passage of blood from patients with acute leukemia, the allantoic fluid of the inoculated embryos was heated on a water bath to 80°C for 30 and 60 min; the same fluid was treated with 1% formalin. Allantoic fluid at the 15th passage of leukemic blood was heated to 80°C for 40 min and to 100°C for 30 min, and at the 12th passage of filtrate of leukemic brain tissue it was heated to 100°C for 30 min. Allantoic fluid at the 20th passage of leukemic blood was autoclaved for 40 min at 1.5 atmos. Chick embryos were inoculated with this fluid and 4 "blind" passages were carried out, after which the fluid was injected into mice. At the 23rd passage of leukemic blood the allantoic fluid was frozen to -70°C and dried. This preparation was injected into animals 9 days and 2½ months after lyophilization. Allantoic fluid at the 5th and 12th passages of leukemic blood and also at the 10th passage of leukemic brain filtrate and the 3rd passage of filtrate of normal brain were added, in proportions of 1:1 or 3:1, to a 5% suspension of human red cells (from a donor of Group I (0), Rh negative) in physiological saline. The mixture was kept at a temperature of 4°C for 3 hours. The red cells were then precipitated by centrifugation two or three times and washed with cold physiological saline. The precipitate of red cells was made up to a 5% suspension in physiological saline.

To find out whether adsorption of "leukemic factor" on red cells could take place, we also studied its specific antigenic properties, using L. A. Zil'ber's test of anaphylaxis with desensitization.

TABLE 1

The Properties of "Leukemia Factor" After Passage through Chick Embryos

Preparation injected	Age of mice	Site of injection of preparation	Number of experimental mice	No. of mice surviving until first sign of leukemia	Average latent per. of leukemia development in	Number of leukemias developing
Allantoic fluidat the 6th passage of leukemic blood (crude)	2—8 days	Subcutaneously	79	45	1,5	9
	1 month	Into the spleen	21	4	4,0	2
The same fluid heated to 80°C	2—10 days	Subcutaneously	61	32	—	0
	1 month	Into the spleen	21	16	—	0
The same fluid treated with 1% formalin	3 days	Subcutaneously	21	19	—	0
	3 months	Into the spleen	20	16	—	0
Allantoic fuid at the 20th passage of leukemic blood, autoclaved	1 month	The same	62	41	5,8	7
The same fluid heated to 80°C	1 »	» »	41	6	10,0	1
The same fluid heated to 80°C	1 »	» »	32	7	—	0
Allantoic fluid at the 20th passage of leukemic blood, autoclaved	25 days	Subcutaneously	22	5	—	0
Allantoic fluid at the 23rd passage of leukemic spleen	1 month	The same	33	6	—	0
	1 »	Into the spleen	20	2	2,0	1
	1 »	Into the thymus gland	22	11	1,0	4

TABLE 2

Adsorption of "Leukemic Factor" on Red Blood Cells

Preparation injected	Age of mice	Mode of injection of preparation	No. of expt. mice	No. of mice surviving to develop leukemia	Avr. latent per. of leukemia development in mo.	No. of leukemias developing
Allantoic fluid at the 5th passage of leukemic blood; adsorption on red cells	1— 10 days	Subcutaneously	32	6	4.5	2
The same, heatd to 100°C	10 days	» »	10	3	—	0
Allantoic fluid at the 5th passage of leukemic blood; adsorption on red cells	1 month	Intraperitoneally	20	11	1.7	2
Allantoic fluid at the 10th passage of a filtrate of leukemia brain; adsorption on red cells.	1 »	The same	20	18	1.7	2
Allantoic fluid at the 12th passage of normal blood; adsorption on red cells	1 »	» »	20	12	—	0
Allantoic fluid at the 3rd passage of filtrate of normal brain; adsorption on red cells	1 »	» »	20	12	—	0

All the preparations described above were injected into mice of CC_{57}, C_{57}, C_3HA and unidentified strains, to test their leukemia-producing activity. They were injected into the animals subcutaneously (usually in the

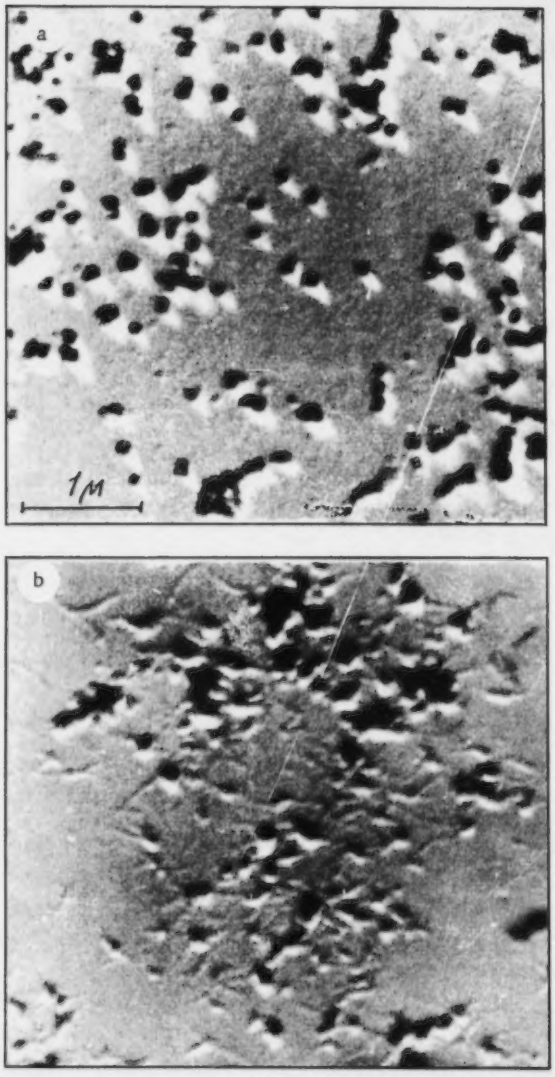

Fig. 1. Allantoic fluid under the electron microscope. a) From a chick embryo inoculated with leukemia; b) from a healthy chick embryo.

case of newborn mice), directly into the tissue of the spleen and directly into the tissue of the thymus gland of mice aged 25-30 days.

Blood cultures were taken from the experimental mice in order to detect any paratyphoid infection which could give a leukemoid reaction. In only one mouse (which had received autoclaved allantoic fluid) was Salmonella Gärtneri found; no paratyphoid bacteria were discovered in the remaining cases.

The animals which died or were killed were examined histologically and cytologically with great care, in order to make a diagnosis of true leukemia. In case of necessity (7 mice) the systemic diseases produced in the mice were transplanted into other mice of the same strain to differentiate true leukemias from leukemoid reactions.

EXPERIMENTAL RESULTS

As seen from Table 1, crude allantoic fluid at the 6th passage of leukemic blood on embryonic membranes of chicks produced leukemia in 22.4% of mice. The same allantoic fluid, when heated to 80°C or treated with 1% formalin, completely lost its ability to produce hemocytoblastoses or myeloses in mice.

When injected into the spleen of mice at the 15th passage of leukemic blood, crude allantoic fluid led to the appearance of leukemia in 17% of the mice, after an average latent period of development of 5.8 months. The same allantoic fluid, if heated to 80°C, caused leukemia in only one mouse of every 6 which survived for 10 months from the beginning of the experiment (the period of development of leukemia); heating the allantoic fluid to 100°C inactivated it. Heating to 100°C also destroyed the leukemia-producing activity of allantoic fluid at the 12th passage of a filtrate of leukemic brain.

"Leukemic factor" is also inactivated by autoclaving. Autoclaved leukemic allantoic fluid was subcultured 4 times by blind passage on embryonic membranes of chicks. Inoculation of mice with allantoic fluid at the 4th passage of autoclaved "leukemic factor" did not cause the appearance of hemocytoblastoses nor myeloses.

Human "leukemic factor" is thus inactivated by high temperatures and by 1% formalin.

As we know, viruses possess considerable resistance to cold. In a frozen state, suspensions of viruses remain active for a long time: at a temperature of -70°C viruses survive for a year and more; and in the dried form (after freezing) preparations of viruses do not lose their powers of infection for several years.

We also investigated the leukemia-producing activity of frozen and dried preparations of leukemic allantoic fluid.

It was shown that frozen and dried allantoic fluid at the 23rd passage of leukemic blood kept its activity for at least 2½ months. Of 19 mice which survived to show the first signs of leukemia, leukemia was observed in five, with an average latent period of development of 1½ months. Leukemia was found in mice receiving injections of the preparation directly into the tissue of the spleen or thymus gland (when the latter method of injection of the preparation was used, very malignant hemocytoblastoses developed).

Many viruses have the ability to undergo adsorption on red blood cells. We studied the ability of human "leukemic factor" to undergo such adsorption by means of a biological test of the specific leukemia-producing activity of the preparations obtained and by the anaphylaxis with desensitization reaction.

As seen from Table 2, allantoic fluid at the 5th and 12th passages of leukemic blood, adsorbed on red cells, possessed leukemia-producing activity and caused leukemia in 23% of mice with an average latent period of development of 3.1 months. Allantoic fluid at the 10th passage of a filtrate of leukemic brain, adsorbed on red cells, was also active (leukemia was observed in 2 of the 18 mice which survived the time for the first case of leukemia to develop).

In control experiments in which human red cells were added to allantoic fluid at the 12th passage of normal blood or at the 3rd passage of a filtrate of normal brain, no leukemia was observed.

The results of these experiments indirectly confirm the results of the study of the specific antigenic properties of the "leukemic factor" adsorbed on the red cells. In 3 guinea pigs of six sensitized to leukemic allantoic fluid at the 14th passage and adsorbed on human red cells, an anaphylactic reaction (accessed +) developed in response to an assaulting injection of leukemic allantoic fluid at the 22nd passage.

Allantoic fluid at different numbers of passages of leukemic and normal blood was studied under the ÉM-3 electron microscope by G. I. Abdeev. The specimens were prepared as follows: one drop of allantoic fluid, centrifuged at 6000 rpm, was placed on a collodion film; excess fluid was removed; after drying, the film was washed 5-6 times with distilled water; the preparations were shadowed with nichrome at an angle of 15°. On examination of the "leukemic" films (Fig. 1 a), at some passages round bodies were found, usually 100-125 mμ in diameter, and sometimes grouped in clusters. Similar particles were also encountered in control films, but they were few in number and rarely seen (Fig. 1 b).

To conclude this account of the concrete results of the experiments we must emphasize particularly that all the leukemias produced in these experiments were, by their morphological structure, hemocytoblastoses or myeloses. Besides these, we often noted the appearance of extensive systemic lesions of reticular tissue, from time to time indistinguishable under the microscope from true reticuloses (hemohistioblastoses). Under these circumstances areas of systemic hyperplasia of reticular tissue were found not only after injection of the "leukemic factor" but also in the control experiments. However no hemocytoblastoses not myeloses were present in the latter.

The facts described suggest that reticuloses may develop in mice as a result of various nonspecific agents. It has still to be explained whether these "reticuloses" are true malignant neoplasms (hemohistioblastoses) or whether they are severe systemic reactions of the reticular tissue. This interesting and formidable problem will be the subject of a special study. So far as human "leukemia factor" is concerned, it possesses the specific property of causing hemocytoblastoses and myeloses in experimental animals.

Judging by the results of the investigations which we have described, the human "leukemic factor", like the majority of the known viruses (including the tumor-producing viruses), is inactivated by the action of high temperatures and of 1% formalin.

At the same time it evidently is adsorbed on red blood cells and is preserved for at least 2½ months by freezing to -70% and subsequent drying.

SUMMARY

The author presents results of examination of the properties of human "leukemic factor" cultured on the chorion allantoic membrane of developing chick's embryos. It was established that "leukemic" allantoic fluid loses its leukosogenic activity in action of high temperatures and its treatment with 1% formalin solution. Lyophilized preparations of "leukemic" allantoic fluid (which was kept in dried condition up to 2½ months) caused

the appearance of myeloid leukemias and of hemocytoblasts in mice (especially when administered into the thymus gland). Leukemic allantoic fluid adsorbed on human erythrocytes possessed leukosogenic activity. The preparations of "leukemic" allantoic fluid when examined microscopically were found to contain globular bodies 100-125 mμ in size (such particles were rarely revealed in control preparations and were few in number).

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EXPERIMENTAL BIOLOGY

TISSUE CELL CHANGES IN HUMAN LYMPHATIC GLAND EXPLANTATES

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Accounts of changes in human lymphatic gland tissues during explantation are very scanty; they are limited in fact to a series of papers, already quite old, which appear in various existing reviews [7, 9, 17] and monographs [12]. The authors have cultivated particles of lymphatic glands in order to solve certain specific problems (movement, rate of migration of lymphocytes and so on), without dealing at all with the growth characteristics of the cultures, the relationships between their component cells, and the ability of these cells to undergo change and differentiation. As test objects they used only normal lymphatic glands of rabbits [7, 8], apart from only one investigation devoted to culture of material from lymphatic glands of human fetuses [11].

It is not yet established with certainty whether the lymphocyte of the blood and lymph of different animals and man is a cell of low differentiation capable of progressive development, or whether it must be regarded as a cell with a specialized and diminishing power of conversion into other cell forms [1, 2, 4, 5, 10, 13-16, 19, 20]. This question has frequently been discussed but remains far from solved. When working with explantation of a leucocyte film the possibility of confusion by conversion of lymphocytes and monocytes cannot be excluded [24]. Explantates of lymphatic glands have the advantage over cultures from leucocyte films that monocytes, numerous in the latter, are practically absent.

The study of the hemopoietic organs, especially the lymphatic glands of the adult human subject, appeared therefore to be of great interest.

EXPERIMENTAL METHOD

The material cultivated consisted of fragments of the cortex of lymphatic glands, obtained from patients aged from 27 to 74 years, admitted to the First and Second Surgical Clinics of the Institute of Oncology. Cultivation took place in flasks. The solid phase was composed of heparinized chick plasma, Tyrode fluid and chick embryonic extract; the fluid phase – human serum to which was added Tyrode fluid. In all we carried out 24 series of experiments with 12 to 16 cultures in each. The cultures were studied for 45 days, whether living or whether at a standstill. Fixation was by 10% formalin, Carnoy's fluid and Zenker's formol. Staining was by Carazzi's or Heidenhain's hematoxylin, and by azure-eosin. To demonstrate argyrophilic fibers, the sections were fixed and impregnated with silver nitrate by Foot's method. Lipids were stained by Sudan III and Nile blue sulfate. Parallel studies were carried out on the original material used for the cultures. The explantated fragments usually consisted of cells of the reticular stroma with argyrophilic fibers, a small number of fibroblasts in the thickness of the trabeculae and capsule, and also along the course of the vessels, a large number of free lymphocytes (small and of medium size), and a few lymphoblasts distributed among the loops of the reticular stroma. Sometimes plasma and lipoid cells were encountered.

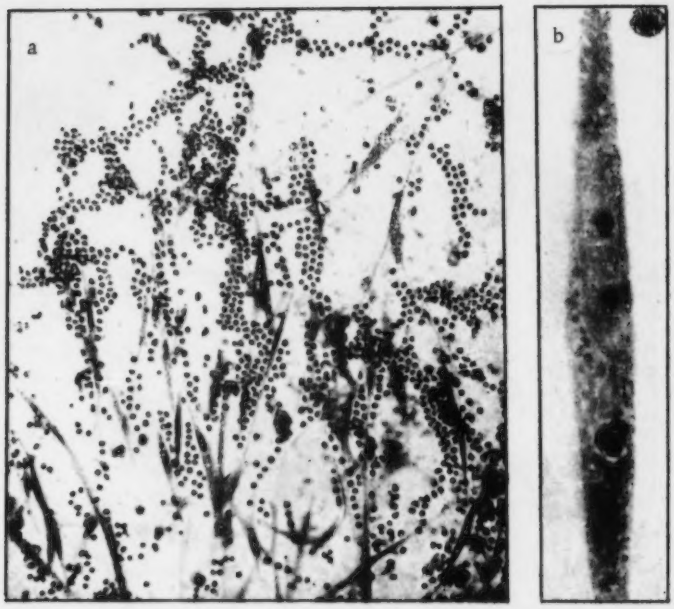

Fig. 1. Reticular cells and collections of lymphocytes in the zone of growth of an 8-day culture of the lymphatic gland of a 66-year old woman.
a) Total preparation. Formalin. Carazzi hematoxylin. Magnification 200 x. Basophilic granules and a phagocytosed lymphocyte in the cytoplasm of a reticular cell; 8-day culture of the lymphatic gland of a 66-year old woman; b) total preparation. Formalin. Caracci hematoxylin. Magnification 1300x.

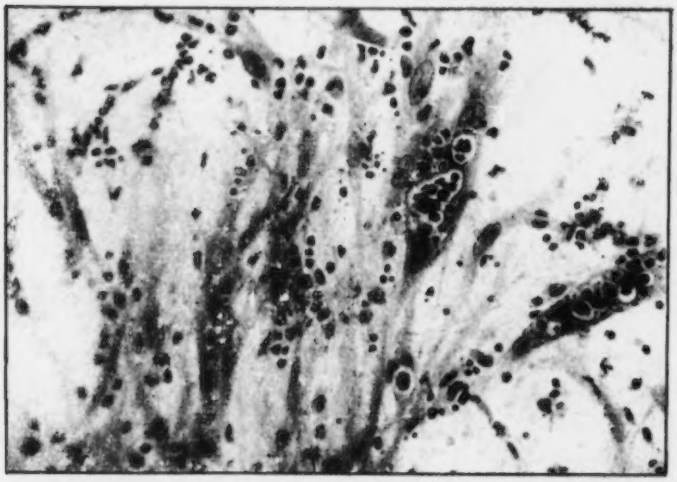

Fig. 2. Growing reticular syncitium with phagocytosed lymphocytes; 8-day culture of the lymphatic gland of a 42-year old woman; total preparation. Formalin. Carazzi hematoxylin. Magnification 1300x.

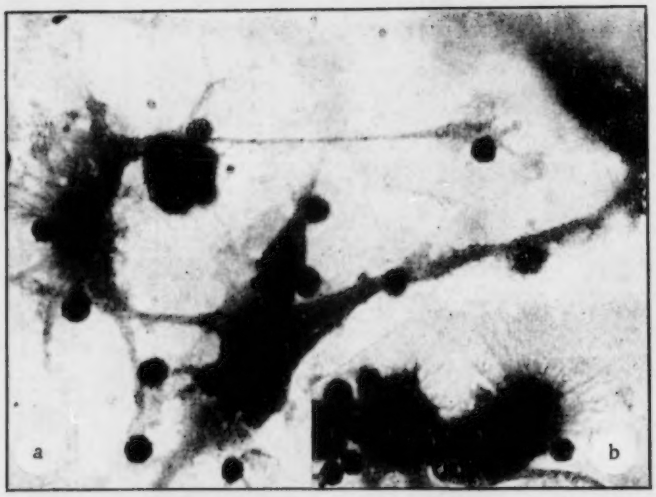

Fig. 3. Formation of "vesicles" (a) and axopodia (b) by reticular cells with phagocytosed lymphocytes; 4-day culture of the lymphatic gland of a 48-year old woman. Total preparation. Formalin. Carazzi hematoxylin. Magnification 1250x.

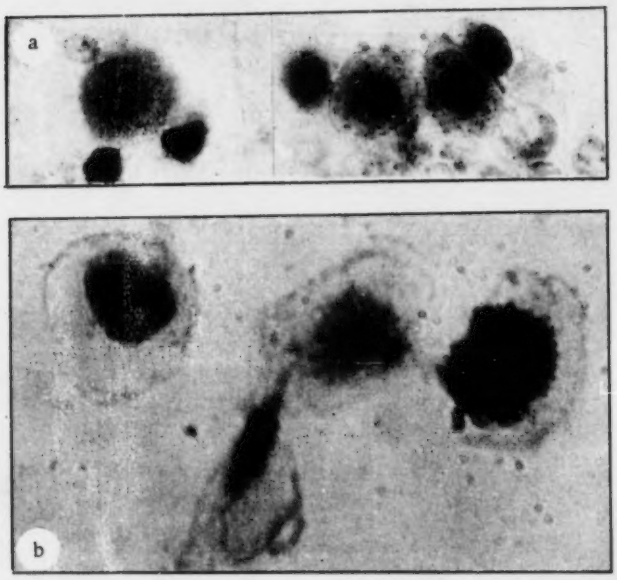

Fig. 4. Mitoses in polyblasts; 9-day culture from the lymphatic gland of a 35-year old woman (a); total preparation. Formalin. Carazzi hematoxylin. Magnification 1300x. Epithelioid cells with lipoid inclusions; 9-day culture from the lymphatic gland of a 35-year old woman (b). Total preparation. Formalin. Carazzi hematoxylin. Sudan III. Magnification 600x.

EXPERIMENTAL RESULTS

Twenty-four hours after explanation, the tissue fragment is surrounded by a wide zone of migration of lymphocytes. At later periods of cultivation the number of these cells in the zone of migration shows a marked increase; at the same time death of a high proportion of them takes place. At the end of the second and beginning of the third day, cells of the reticular stroma — spindle-shaped, stellate or having numerous processes — appear in the fibrin coagulum. Proliferation of these cells is sometimes accompanied by intensive fibrinolysis. They multiply intensively by mitosis, and after 5-6 days produce a quite extensive zone of growth, very reminiscent of the zone of growth in cultures of loose connective tissue [6, 27].

Alongside the growth of fibroblast-like reticular cells, proliferation of the relatively few fibrocytes of the trabelulae also takes place.

The structure and character of the zone of growth of connective and reticular tissues have much in common, which from time to time makes the picture extremely difficult to interpret. In such cases the criteria are the well marked syncitial structure of the reticular tissue and certain morphological and physiological properties of its component cells. It is also very difficult to distinguish the endothelium of the sinuses from the reticular stroma and the endothelium of the vessels from the fibroblasts of the trabeculae and capsule. This difficulty, which was previously mentioned by Grossmann [22], is particularly evident in cases of mixed growth. The difficulty in distinguishing between these cells depends on the properties, mentioned above, of the stroma of the lymphatic gland, and also on the fact that the endothelium of the vessels contains highly friable structures in addition to its complex membranes [18].

One very often observed characteristic feature of lymphoid tissue in tissue culture is the intimate relationship between the cells of the reticular stroma and the lymphocytes. The latter, situated in the loops of the reticular syncitium and in close contact with its cells, remain viable for a long time. Thanks to some influence (possibly of the nature of chemotaxis or thigmotropism) many of these remain for the whole time in direct contact with the reticular cells. Some of them lie so closely up against them that they appear to be adherent to the surface of the body or processes of these cells (Figs. 1, a and 2). When they migrate in the fibrin, the lymphocytes often form characteristic groups, shaped like narrow bands, round collections or garlands with branches in the form of trees (see Fig. 1, a). Structures to some extent similar were described by Hueper and Russell [23] in cultures of white blood cells as capillary-like formations. However, a careful study of these collections of lymphocytes makes it clear that they have nothing in common with endothelial vascular tubes but show their own distinctive nature and pattern of aggregation with each other, and their association with the cells of the reticular syncitium.

Reticular cells, which together with lymphocytes account for the bulk of the zone of growth in cultures of lymphatic glands, have a varied external appearance and a varied structure, which depend not only on the properties of the tissue itself but also on the conditions of its existence. Reticular cells growing in a fibrin coagulum are spidle shaped or have numerous processes. They are most commonly slightly elongated and have an outward resemblance to fibrocytes, which gave certain authors [7, 8, 11] grounds for identifying them with the fibrocytes of connective tissue. Reticular cells have a well-outlined cell body, a pale nucleus, poor in chromatin and usually with 1 or 2 nucleoli, and feebly basophilic protoplasm. Often it shows numerous vacuoles and basophilic granules (Fig. 1, b) and also a small number of droplets of fat.

Under certain conditions (a large quantity of decomposition products in the zone of growth, liquefaction of fibrin) some of the reticular cells lose their connection with each other and are converted into free, ameboid macrophages. When they migrate along the film into the cavity of liquefaction, the reticular cells show great variation in size and shape. The majority of them have feebly basophilic cytoplasm. This has a well-marked foamy structure, with numerous processes and vesicular formations with translucent, watery contents (Fig. 3, a).

In other cases, in a state of retraction, the cytoplasm of the cells of the reticular syncitium sometimes forms numerous thin, pale processes which resemble axopodia (Fig. 3, b). Both the attached and free reticular cells possess well-marked phagocytic properties and they vigorously engulf dead and living lymphocytes. The lymphocytes are situated in the loops of the reticular syncitium and as a rule are surrounded by a light rim — a digestive vacuole (see Fig. 2). In the course of time the phagocytic properties of the reticular tissue in the cultures grows weaker, although they are sometimes preserved until late periods of cultivation. Mitoses are often seen in the actively phagocytosing cells. Multinuclear giant cells, developing from reticular cells, are rarely

encountered; in the whole of our material we were able to find them in 3 series of experiments, but not in every culture and in small numbers. Multinuclear cells developed mainly in the liquefaction cavities on the surface of the film, but they were also to be found in the fibrin coagulum. In isolated cases the multinuclear cells attained a considerable size and contained 10 or more nuclei.

Within a day or two of explantation the lymphocytes begin to undergo changes. Being the least stable cells in these cultures, they very rapidly undergo necrobiotic changes in large numbers. The fate of the lymphocytes which remain viable is as a rule varied: some of them remain visibly unchanged until the last days of observation, while others — mainly those situated between the reticular cells and on their surface — undergo further progressive changes. These changes in the lymphocytes begin on the 5th-6th day of cultivation. Some of them become round and larger in size. The basophilia of their cytoplasm becomes more intense and their nucleus becomes excentrically situated and acquires the characteristic structure of plasma cells. The plasma cells seen in the cultures on the 6th-8th day after explantation are undoubtedly formed there, for they are only rarely found in the original material and furthermore they die in the course of the first 2-3 days.

Development of plasma cells is observed in few cultures (1-2%). The bulk of the lymphocytes develop into macrophages. In both the unchanged lymphocytes and in the macrophages formed from lymphocytes many mitoses are seen. Some of these cells become binuclear as a result of amitotic division of the nucleus. Sometimes fragmentation of the nucleus is seen. The increase in the number of macrophages in the zone of growth takes place on account of the continuing conversion of lymphocytes and of mitotic division. Mitoses are especially numerous in the 8-9-day cultures (Fig. 4, a).

On staining with Sudan III a large number of small droplets of fat are shown in the cytoplasm of the completely viable and intensively proliferating macrophages. The subsequent fate of the macrophages which form differs: some of them die after a brief existence, others, depending on conditions, are converted into other cell forms. A very small number of macrophages remains unchanged for a considerable length of time.

Conversion of macrophages into fixed cells of the fibroblast type is seen rarely (6-8%). In cultures where this occurs, a thin zone of growth forms, almost identical with that forming in connective tissue explantates. However the fibroblast-like cells, developing from lymphocytes, under certain conditions (cooling, mechanical injury and so on) become round again and become converted into free, round macrophages. The formation of multinuclear giant cells (by fusion) from macrophages is observed still more rarely. Often these giant cells do not differ from those formed from the cells of the reticular stroma and they appear in the central fragment and in the zone of growth, not only around foreign bodies but also where these are absent. Very often the macrophages are converted into cells which were described by a number of authors under the name of "epithelioid cells" [21, 25, 26]; they develop in those cases where the macrophages are spread out over the film in the cavity of liquefaction. Differing widely in their shape and size, the "epithelioid cells" retain to only an insignificant degree their powers of phagocytosis. The protoplasm of their bodies is always sharply divided into a central, endoplasmic part, strongly basophilic and containing the nucleus and a wide, transparent, almost colorless but clearly defined ectoplasm (Fig. 4, b). In the endoplasmic part are sometimes found solitary basophilic granules and a varying number of droplets of fat. Sometimes the "epithelioid cells" form long, slender and straight processes which connect them to each other.

Comparison of our own findings with those in the literature shows that the lymphocytes of the blood and lymphatic glands undergo similar changes in culture. Both develop into macrophages, fibroblast-like cells and "epithelioid cells." However in cultures of lymphatic glands, as also during aseptic inflammation [3], the conversion of lymphocytes into macrophages, "epithelioid cells" and fibroblast-like cells takes place more slowly and in much smaller numbers. In contrast to explantates from leucocyte films [4], "epithelioid cells" in cultures from lymphatic glands were never combined with each other inside epithelial-like membranes and they could never be seen to form multinuclear giant cells. In the conditions of our experiments the lymphocytes of the zone of growth were not converted into cells identical with connective tissue fibroblasts and after cultivation for 30-40 days no fibrous intercellular material was formed. The latter fact is in complete agreement with the findings of A. I. Zhudina [4], who studied the changes in the human white blood cells in tissue cultures. However, by itself it is far from opposing the fundamental views of A. A. Maksimov and of other workers, who consider that the lymphocytes are cells of low differentiation, capable of progressive development in various directions. The results which we have obtained is new evidence of the great powers of development of the lymphocytes of adult human lymphatic glands.

In cultural conditions a large part of the reticular cells retain their sessile character and resemble fibroblasts. In this condition, however, they are actively phagocytic and, at various stages of cultivation, they are converted into macrophages which is not characteristic of the fibrocytes of connective tissue, according to the view prevailing at the present time.

SUMMARY

The change of lymphocytes into macrophages, fibroblastlike cells and "epitheliod" elements was observed in culturing the tissue of human lymph node. Lymphocytes were not transformed into elements identical to fibrocytes of connective tissue and did not form the fibrous intercellular substance in 30-40 days of culturing. The majority of the reticular cells preserved its stationary nonameboid character and resembled fibroblasts. They exhibited an actively phagrocytic function and were transformed into macrophages at various stages of culturing.

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HISTOLOGICAL INVESTIGATION OF THE RESULTS OF TENDON REPAIR IN RABBITS USING VARIOUS PLASTIC MATERIALS

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The problem of the plastic repair of tendon defects is of great theoretical and practical interest. One of the main factors towards finding a successful solution of this problem is the choice of the most suitable raw material for the tendon graft. As yet there is no unanimity on this question. There are differences of opinion on the value of the simplest method of repair of a tendon defect, the alloplastic. There is no full agreement over comparison of the results of autoplastic repair of tendon defects with strips of fascia and tendon autografts, nor over the use of homografts for this purpose.

EXPERIMENTAL METHOD

Experiments were carried out on rabbits in which a defect in the tendo Achillis was repaired in four ways (see Table).

The tendo Achillis was exposed in the rabbits and from it was resected a piece about 2 cm long, after which the resulting defect was repaired with various plastic materials. In the first series of experiments silk thread was used for this purpose and was stretched between the ends of the tendon with known tension. In the 2nd series a defect of the same size was repaired with a homoplastic graft (2 cm long) from a rabbit. In the 3rd series the defect was repaired with strips of fascia lata from the thigh of the same animal, which was folded into a sleeve and sutured to the ends of the resected tendon. In the 4th series the defect was repaired with a tendon autograft taken from the other limb of the same rabbit. In all cases the grafts were sutured to the ends of the resected tendon by a Cuneo suture with fine (No. 0) silk. After suture of the tendon, the sheath was carefully sutured with fine continuous catgut. The skin was then sutured and the limb immobilized in a plaster of Paris cast for 14 days. Material taken for histological examination in all the experiments was fixed in 20% formalin (8% formaldehyde), cut on a freezing microtome and only partially embedded in paraffin wax. Sections were stained with hematoxylin-eosin.

EXPERIMENTAL RESULTS

From an analysis of the macro- and microscopic data from the experiments in which the tendon defect was repaired with silk thread, we observed that regeneration of connective tissue occurred along the course of the thread and on the 46th day it had reached 2 mm to become a thin band of scar tissue, adherent to the skin throughout its length; the same picture was observed at later periods — after 4-4½ months.

In our experiments we found degenerative changes in the region of the tendon ends joined by the silk thread. These changes took the form of obliteration of the structure of the collagen bundles and death of the tendon cells. Around the tendon stump and along the course of the silk thread there was new formation of dense fibrous connective tissue with very fine primary collagenous bundles arranged longitudinally and a large number of cells. The silk thread occupied an eccentric position in the regerating tissue and was infiltrated with leucocytes. In places in the dense connective tissue in the area of regeneration there were layers of loose connective tissue.

Number of Experiments and Periods of Observation

Series	Method of repair of defect	No. of experiments	Period of observation (number of days)
1	Silk thread	7	46-220
2	Tendon homograft	12	4-30
3	Fascial autograft	9	22-270
4	Tendon autograft	32	4-360
	Total	60	-

At later periods (after 4-6 months) the newly formed dense connective tissue situated along the course of the silk thread now resembled tendon tissue in its fine structure; on longitudinal and transverse sections the division of this tissue into primary and secondary bundles was clearly seen. Between the primary bundles were arranged cells with elongated nuclei, and between the secondary — layers of loose connective tissue containing blood vessels, nevertheless the structural resemblance found between this regenerating scar tissue and normal tendon (which agrees with the findings of G. M. Voronina and A. M. Gratsianskaya [2]) is still no argument in favor of the success of this method because the scar is unsatisfactory as an organ and it has extensive adhesions to the skin and surrounding tissues.

In cases where tendon homografts were used, at quite early stages rarefaction of the grafts was observed followed by complete degeneration and disintegration in the majority of cases. One third of our cases showed a relatively satisfactory preservation of the tendon graft. The degree of preservation of the homografts presumably depended on the biological nearness of the donor to the recipient. With a less pronounced immune reaction on the part of the recipient, degeneration in the homograft was also less pronounced and was confined to death of cells alone; the collagen fibers retained their structure. Later on the noncellular collagen tissue was observed to be in process of population by cells of fibroblastic type from the recipient. In these cases we can speak of the partial survival of the homografts. Subsequently these grafts underwent reorganization and partial replacement by dense fibrous connective tissue. However, in these cases an inflammatory reaction took place around the homografts in the form of an infiltration of cells leading to the formation of adhesions between the graft and surrounding tissues, considerably impeding the function of the limb.

Our results with tendon homografting thus do not lead to a favorable evaluation of this method.

In the next series of experiments strips of fascia lata from the thigh were used to repair the tendon defect. In all cases we observed survival of the fascial autografts. In this series of experiments the defect between the tendon stumps was also filled mainly with newly formed connective tissue. Side by side with the formation of a connective tissue scar, however, the partial survival of the transplanted material was observed; in this case the transplanted fascial tissue at first underwent degeneration to some extent, and later it became reorganized into tissue of the tendon type. We observed survival of the fascial autografts in all cases, but on account of the high plastic properties of fascia, extensive adhesions developed with the skin and surrounding tissues, which to a large extent impeded the function of the organ.

In a histological study of the transplanted fascia we were able to make out some degree of degeneration of the fascial tissue from 22 to 26 days after grafting in the form of partial death of the cells and reduction followed by total disappearance, of the transverse fibers. At the same time we observed an increase in the number of longitudinal fibers. The cells were preserved mainly in the peripheral portions of the fascial grafts. In the central portions of the grafts only the nuclei were left of most of the cells and these had disintegrated into clumps of chromatin. On the 40th day after grafting transverse fibers were left only in isolated areas of the transplanted fascia and the bulk of the fibers were longitudinal. Cells were distributed more thickly at the periphery of the layers of fascia. Between the layers of fascia there was marked new formation of dense fibrous connective tissue, often in greater volume than the material originally grafted. On the 70th day after grafting further reorganization

of the fascial autograft was observed, as shown by complete disappearance of the transverse fibers and a continuing increase in the number of longitudinal fibers. After 3 months it was already impossible to distinguish the grafted fascia among the mass of newly formed connective tissue based upon it.

In the opinion of several workers [5, 6], fascia is preserved intact after grafting. Our experimental results do not support this view but on the contrary agree with the findings of P. G. Kornev [4]. From our observation of the survival of a considerable portion of the fascial transplant and its subsequent reorganization, we believe that a fascial sleeve is a satisfactory conductor of regenerating tendon tissue, yet nevertheless the results of the experiments which we carried out must be regarded as unsatisfactory, since gross adhesions are formed under these circumstances with the surrounding tissues, impeding the function of the limb.

In the 4th and main series of our experiments we performed autoplastic tendon grafting. It must be pointed out in the first place that macroscopically the autografts developed a matte appearance after the first few days, losing their silver gloss, characteristic of the normal tendon. Accretion of the graft to the bed was observed only 16 days after transplantation. Until the 50th day the grafts preserved their initial length and diameter, and then a small increase was observed in the length of the grafts and some increase in their diameter. At 9 months the characteristic sheen began to appear on the grafts and one year after transplantation the tendon autograft shone like a normal tendon. In this respect the results of our experiments agree with those of N. Z. Tomilova [7], who observed the appearance of the characteristic silver sheen in a tendon autograft 9 months after transplantation. However, in contrast to our findings, this worker points out that the tendon autografts gradually became stretched and thinned so that their functional value diminished. This opinion of N. Z. Tomilova was challenged by Yu. Yu. Dzhanelidze [3], who considers that the grafts do not stretch and their function, on the contrary, improves in the course of time.

The histological investigations carried out in this series of experiments showed that the transplanted material mainly survives. Only its cells die, mainly those situated in the central areas of the graft. The collagen bundles do not die. In the course of time the collagen tissue becomes enriched with cells, first as a result of proliferation of the cells of the endo- and peritenon and later as a result of division (mainly amitotic) of the tendon cells themselves. During this process of population of the collagen bundles with cells, the bundles of the first order are reduced in diameter, as if by fission. Side by side with this reorganization of the old tendon tissue new formation of young connective tissue is taking place on account of cells of the peritenon of the grafts; the tendon stumps of the graft bed also take part in this process. In this case, then, besides survival of the already existing tendon material, new tendon tissue is formed as a result of regenerative processes. Both donor and recipient take part in these regenerative processes. The newly formed tissue may even exceed the volume of the graft. The reorganization of the grafted material and formation of young tendon tissue take place under identical conditions of stretching. This identity of functional conditions leads to identity of structure and as a result there is formed a tendon whose structure is homogeneous throughout its thickness. In the course of time the differentiation of the tissue of this tendon increases — the collagen bundles of the first order are increased in diameter, the number of tendon cells between them is reduced, these cells become elongated and flattened and their nuclei become rod-like in shape. The layers of endotenon become thinner. To sum up (at the end of one year after grafting) the tissue of the tendon autograft becomes indistinguishable in its structure from the tissue of a normal tendon.

SUMMARY

In replacing tendon of Achilles defects with silk thread and in tendon homoplasty the results were unsatisfactory. In facial autoplasty the transplant partially took and was reconstructed by the tendon type. However, the function of the extremity was disturbed as a result of extensive adhesions of the transplant to the surrounding tissues. The autoplastic transplantation of the tendon tissue resulted in complete restoration of the structure and reestablishment of the function of this organ.

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THE ACTION OF IMMUNE SERA ON THE GROWTH OF THE HEART AND CRYSTALLINE LENS OF CHICK EMBRYOS

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Since 1952 we have put forward from our laboratory the view that immunological relationships play a part in the formative processes in the embryo [1-3]. In this connection we may note with interest the results of experiments in which the action of immune organ antisera on the growth and development of the corresponding organs was studied in the course of embryogenesis. In 1939 Weiss [8] reported that antisera against the liver, kidneys and muscles of the chick, injected into developing hens' eggs, caused a sharp increase in the rate of growth of the corresponding organs in the chick embryos. On the basis of these findings, Weiss [9] put forward the hypothesis that antiorgan antibodies act as a matrix on which is formed the specific proteins of the corresponding organ and thereby stimulate the growth of this organ. Pomerat [7], however, did not confirm Weiss's results. Ebert [4] showed that brain, heart and spleen antisera of chicks depress and do not stimulate the growth of tissues of chick embryos cultivated in vitro. Finally, Flickinger, Levi and Smith [5] were unable to find any specific action of antilens serum on the development of the crystalline lens in chick embryos, both in vitro and in vivo.

The findings concerning the action of immune sera on the growth of the organs of embryos are thus very conflicting and do not permit the drawing of any definite conclusions.

In order to solve this problem, in 1956 we carried out an experimental study of the action of antilens and antiheart sera, injected in various doses into developing eggs, on the growth of the heart and crystalline lens of chick embryos.

EXPERIMENTAL METHOD

We obtained the sera by means of immunization of rabbits with tissue suspensions of cardiac muscle of an adult fowl and crystalline lenses of an adult duck. The sera reacted with homologous antigens in the complement fixation test in a dilution of 1:320 and in the precipitation test in a dilution of 1:10,000. The eggs to receive the injections were from chicks of the "Russian White" breed incubated for five days. The sera were injected into the air sac of the egg, since it was shown in a paper by Freericks [6], that solutions of a number of substances injected into the air sac rapidly penetrate the embryo itself. For this purpose we perforated the eggshell over the air sac and injected the serum through this orifice with a syringe. After the injection the orifice was sealed with paraffin wax and the egg returned to the incubator. Antiheart serum was injected in doses of 0.4, 0.1 and 0.05 cm^3, and antilens serum — 0.4 and 0.1 cm^3. As a control we injected normal rabbit serum in doses of 0.1 and 0.05 cm^3. Some of the eggs were incubated without receiving injections of sera and these were used later for determining the normal weight of the organs. On the 8th day after injection, i.e. on the 13th day of incubation, the eggs were opened up and in each series we determined the weight of each embryo, the weight of the heart, the crystalline lenses and the liver. After weighing, the organs were fixed in Helly's fluid for histological examination. Altogether 104 eggs were used in the experiment.

TABLE 1

Average Weight (in mg) of the Organs of Chick Embryos (13 days of incubation) After Injection of Immune Sera into the Eggs

Name and dose (in cm^3) of serum	Total weight of embryo	Weight of organs		
		heart	crystalline lens	liver
Antiheart 0,4	3920	46,6	3,0	84,7
» 0,1	5350	58,1	3,0	102,6
» 0,05	5055	65,2	3,0	97,5
Antilens 0,4	5298	76,2	2,5	98,1
» 0,1	6430	77,6	3,3	144,1
Normal 0,1	5880	70,4	3,0	114,6
» 0,05	5715	66,0	3,1	103,9
Control (no serum injected)	5500	67,6	3,0	103,3

TABLE 2

Differences in the Average Weight of the Organs of Chick Embryos (13 days of incubation) After Injection of Immune Sera into the Eggs, and that of Control Embryos of the Same Age (as a percentage of the control weight)*

Name and dose (in cm^3) of serum	Change in weight			
	whole embryo	heart	crystalline lens	liver
Antiheart 0,4	<u>−28,7</u>	<u>−31,1</u>	0	−18,0
» 0,1	− 4,5	<u>−14,0</u>	0	− 0,7
» 0,5	− 9,1	− 3,6	0	− 5,6
Antilens 0,4	− 3,7	+12,7	<u>−16,1</u>	− 5,0
» 0,1	<u>+16,9</u>	<u>+14,8</u>	+ 3,5	<u>+39,5</u>
Normal 0,1	+ 6,9	+ 4,2	0	+10,9
» 0,05	+ 3,9	− 2,4	0	+ 6,6

Note: The figures underlined indicate the statistical significance of the change in weight.

* Injection of normal serum stimulated growth of the embryos, hence comparison of the results obtained with the weight of the embryos injected with normal serum led to a greater difference between the experimental and control figures.

EXPERIMENTAL RESULTS

Injection of the sera into the eggs sharply increased the mortality rate among the embryos. Normally this was 14.3% but after injection of the sera the total mortality was 29.7%. The highest mortality rate (68.2%) was observed after the injection of 0.4^3 of antiheart serum.

The gravimetric results are shown in Tables 1 and 2. We subjected the numerical results to statistical treatment by the Fisher-Student method. Differences were considered to be significant if P did not exceed 0.01.

It will be seen from Table 2 that after injection of the antiheart serum in doses of 0.4 and 0.1 cm^3 growth of the heart was sharply retarded. Injection of 0.4 cm^3 of antiheart serum resulted also in inhibition of growth of the embryo. Antilens serum, in a dose of 0.4 cm^3, caused a reduction in the weight of the crystalline lens but had no appreciable effect on the weight of the other organs. It is interesting that antilens serum, in a dose of 0.1

TABLE 3

Mitotic Activity of the Cardiac Muscle of Chick Embryos (13 days of incubation) After Injection of Antiheart Serum into the Eggs

Dose of serum injected, cm^3	Mitotic activity		
	No. of cells in interphase	number of mitoses	mitotic coefficient, %
0.4	2 900	32	1.1
0.1	3 150	103	3.27
0.05	3 100	128	4.13
Control (no serum injected)	3 150	137	4.35

Note. The number of mitoses was counted in 50 fields of vision in 3 embryos of each series.

cm^3, had an obviously stimulating action on the growth of the whole embryo, the heart and liver. At the same time the weight of the crystalline lens was unchanged.

A nonspecific stimulating action on the growth of the embryos was also found on injection of 0.1 cm^3 of normal rabbit serum. Antiheart and normal serum, in a dose of 0.05 cm^3, caused no perceptible changes in the weights of the organs of the embryos. The weight of the liver in both experimental and control embryos varied so greatly that in our opinion it is not a suitable object for studying the action of antisera on the growth of the organs of chick embryos. Histological study of the crystalline lens showed no structural abnormalities after injection of the antilens sera. On the other hand, the heart muscle of the embryos subjected to the action of a large dose of antiheart serum (0.4 cm^3) showed gross damage. Examination of the hearts of this series of embryos showed that they were considerably smaller than normal. Sections showed areas of necrosis side by side with areas of normal muscle. In these places loss of the transverse striation of the muscle fibers is seen; the fibers are friable and broken up into separate fragments. In areas of necrosis can be seen many degenerating cells with pyknotic nuclei.

A count of the mitoses in different portions of the heart showed (Table 3) that antiheart serum depresses mitosis in cardiac muscle. The fall in the number of mitoses is greater the larger the dose of antiheart serum. Injection of 0.05 cm^3 of antiserum did not alter the number of mitoses. It is important to point out that after injection of 0.4 cm^3 of antiheart serum, side by side with large areas in the heart where mitoses were encountered extremely rarely other areas were observed in which the number of mitoses was even higher than normal; here there may be almost five times as many mitoses as normally: in these areas there was one mitosis on the average to 4-5 nuclei in the stage of interphase. It is not yet possible to account for the increase in mitotic activity in the individual areas of the cardiac muscle in embryos treated with antiheart serum. Further research will undoubtedly be required to shed light on this interesting phenomenon.

SUMMARY

Thus the injection of antiheart and antilens sera, in a dose of 0.4 cm^3, resulted in our experiments in a considerable loss of weight of the corresponding organs of chick embryos. Antiheart serum, in this dosage, also caused retardation of growth of the whole embryo. Histological examination revealed necrotic areas in the cardiac muscle and a sharp fall in mitotic activity in this tissue. Injection of 0.1 cm^3 of antiheart serum led to loss of weight of the heart but did not affect the growth of the other organs. Antilens serum, in a dose of 0.1 cm^3, stimulated growth of the embryo and the individual organs, with the exception of the lens. A nonspecific stimulating action on the growth of the embryos was also shown by injection of 0.1 cm^3 of normal rabbit serum.

The results of this investigation show that organ-specific antibodies are capable of a specific action on the growth of the corresponding organs.

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A COMPARATIVE STUDY OF THE ANTIGENIC PROPERTIES OF THE CRYSTALLINE LENS IN VERTEBRATES AND INVERTEBRATES

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Uhlenhuth [22] demonstrated the similar antigenic properties of the crystalline lens in various vertebrate animals. According to this worker, the crystalline lenses of mammals, birds and amphibia contain identical proteins (antigens), which are also present in traces in the crystalline lenses of fishes. He considered that this phenomenon was the result of the identical function of the crystalline lens in vertebrates. These findings have been confirmed by several workers [1, 5, 7, 10-16, 20 and others].

Wollman, Gonzalez and Ducrest [24] used the precipitation reaction to study the antigenic properties of the crystalline lenses of the Cephalopoda and of vertebrates. These workers reported that the antilens serum of the octopus (Octopus vulgaris) did not react with antigens of the crystalline lenses of fishes and mammals, and that antilens sera of fishes and mammals did not react with antigens of the crystalline lens of the octopus. Nevertheless Tutui [21] showed that positive precipitation and complement fixation reactions were not observed only when the antigens used in the tests or in preparing the antisera consisted of extracts of the crystalline lenses of very unrelated species of animals (for example ox and cuttle-fish). If the difference between the species was less, the reaction was stronger and clearer.

Thus the problem of the similarity or difference in antigenic properties of the crystalline lenses of the vertebrates and invertebrates has not yet been finally solved, and accordingly we decided to carry out an investigation ourselves.

EXPERIMENTAL METHOD

We studied the antigenic properties of the crystalline lenses of birds (chick, goose), mammals (guinea pig), reptiles (grass snake, Natrix natrix), amphibia (frog, Rana ridibunda), fishes (cod, Godus callarias) and cephalopod molluscs (calamary, Ommatostrephes sagittatus).

In the first series of experiments we studied the antigenic properties of the crystalline lenses of vertebrates by means of the ring precipitation test [5]. Antisera were obtained in rabbits against the water-soluble antigens of the crystalline lens of the chick, by the method suggested by P. N. Kosyakov [6]. The antisera reacted with antigens of the lens of the chick in dilutions of the latter of 1:40,000. These antisera were highly specific and did not react with saline extracts of other organs nor with the blood serum of the chick.

In the second series of experiments we studied the antigens of the crystalline lenses of vertebrates by means of the precipitation test in agar in capillary tubes.

In the third series of experiments we made a comparative study of the antigenic properties of the crystalline lenses of vertebrate birds and fishes and invertebrates (cephalopod molluscs), using the anaphylaxis reaction in guinea pigs [4]. The guinea pigs were sensitized subcutaneously with suspensions of formalized (in 8% formalin)

TABLE 1

Results of the Ring Precipitation Test between Rabbit Antiserum (No. 3261) to the Crystalline Lens of the Chick and Antigens of the Lenses of Various Species of Animals

Antigens of the lens of	Dilution of antigens							
	1:500	1:1 000	1:2 500	1:5 000	1:10 000	1:20 000	1:40 000	1:80 000
Chick	++++	+++	+++	+++	++	+	+	—
Goose	++++	+++	+++	++	+	+	—	—
Guinea pig	+++	+++	++	++	+	+	—	—
Grass snake	+++	+++	++	++	+	+	—	—
Frog	+++	+++	++	++	+	—		
Cod	+++	++	+	+	—			

Note. + + + + dense ring, formation of a precipitate; + + + well marked ring; + + marked ring; + slight ring; - no ring.

TABLE 2

Results of the Precipitation Reaction in Agar between Rabbit Antiserum (No. 3261) to the Crystalline Lens of the Chick and Antigens of the Lenses of Various Species of Animals

Antigens of the lens of	Number of rings of precipitate			
	antiserum to the lens			Normal rabbit serum (No. 3261)
	dense rings	slight rings	total number of rings	
Chick	4	3	7	0
Goose	4	1	5	0
Guinea pig	2	2	4	0
Grass snake	3	0	3	0
Frog	1	1	2	0
Cod	0	Very slight clouding of the agar	0	0
Chick's liver	0	0	0	
Chick's heart	0	0	0	
Chick's brain	0	0	0	

lens tissue of the chick, cod and calamary. The lens tissue was carefully washed free from formalin in tap water. On the 21st day after sensitization, all the guinea pigs were injected intravenously with saline extracts (1:10) of nonformalized lens tissue of the chick or cod. These extracts were also injected intravenously into 4 nonsensitized guinea pigs (technical control). As a further control we used extracts (1:10) of the liver of the cod and chick, which were injected into guinea pigs sensitized by antigens of lens tissue of the chick, cod and calamary.

EXPERIMENTAL RESULTS

It was shown by means of the ring precipitation test with antisera to antigens of the crystalline lens of the chick (Table 1) that the lenses of all the vertebrates studied (chick, goose, guinea pig, grass snake, frog and cod) possess similar antigenic properties. Saline extracts of the lens tissue of the chick reacted in a dilution of 1:40.000,

TABLE 3

Anaphylaxis Reaction in Guinea Pigs Sensitized to Crystalline Lens Tissue of the Chick, Cod and Calamary, in Response to the Injection of an Extract of the Crystalline Lens Tissue of the Cod or Chick

	Sensitization. Antigen-suspension of crystalline lens tissue (does 5 mg)	Assaulting injection. Antigen-extract of crystalline lens tissue of the cod (dose 500 mg/reaction)	Sensitization. Antigen-suspension of crystalline lens tissue (dose 5 mg)	Assaulting injection. Antigen-extract of crystalline lens tissue of the chick (dose 500 mg/reaction
	Chick	+	Chick	++++
	»	++	»	+++
	»	+	»	+++
	Cod	+++	Cod	+
	»	++	»	+
	»	+++	»	+
	Calamary	++	Calamary	—
	»	+	»	+
	»	++	»	—
		Injection of extract of cod liver tissue (dose 500 g/reaction)		Injection of extract of chick's liver tissue (dose 500 mg/reaction)
Control	Chick	—	Chick	-
	»	—	»	—
	Cod	—	Cod	—
	»	—	»	—
	Calamary	—	Calamary	—
	»	—	»	—
		Injection of extract of crystalline lens tissue of the cod (dose 500 mg/reaction)		Injection of extract of crystalline lens tissue of the chick (dose 500 mg/reaction)
	No sensitization produced (technical control)		No sensitization produced (technical control)	—
	The same	—	The same	—

Note. + tremor, running nose and eyes, shaggy fur, dyspnea, slight fall of temperature; + + the same signs more strongly expressed; the guinea pig often sneezes; + + + the same signs more strongly expressed; convulsions, cough, animal lies on its side but survives; + + + + all signs expressed very strongly, animal dies; - no signs of anaphylactic shock.

and extracts of the lens tissue of the goose, guinea pig and grass snake — in a dilution of 1:20,000, frog — 1:10,000 and cod — 1:5000. The results of these experiments show that the further apart the animals are in their position in the system, the less the antigenic similarity of their crystalline lenses.

The results obtained by the ring precipitation test do not show sufficiently clearly, however, the identity of or difference between the antigenic properties of the crystalline lenses of different animals. We thought that this was best demonstrated by the precipitation reaction in agar. As a result of a large number of experiments it was shown (Table 2) that during the interaction of antiserum to the antigens of the crystalline lens of the chick with homologous antigens, 7 rings of precipitate are formed (4 dense and 3 slight), during the interaction with antigens of the lens of the goose — 5 rings (4 dense and 1 slight), with antigens of the lens of the guinea pig — 4 rings (2 dense and 2 slight), with antigens of the lens of the grass snake — 3 dense rings, with antigens of the frog's lens — 2 rings (1 dense and 1 slight) and with antigens of the lens of the cod — 1 very slight clouding of the agar.

If, however, as antigens were used extracts from other organs of the chick (heart, liver, brain), no rings of precipitate were formed in the agar. From the results obtained it can be assumed that there are two different types of organ-specific antigens: organ-specific antigens common to the same organ in different species of animals, and organ-specific antigens which are characteristic for that particular organ in the particular species of animal alone. It may be thought that the presence of common organ-specific antigens in the organs of different species of animals is associated with the common function of these organs and with the family relationships between the different species of animals from the evolutionary aspect, whereas the presence of antigens which are both organ-specific and species-specific is associated with the peculiarities of the function and structure of the organ in the given species of animal.

The results of the second series of experiments show that the more closely related the animals are to each other, the higher the content of common organ-specific antigens in their crystalline lenses and also that the lenses, and also that the lens of the animals of the group Amniota (mammals, birds and reptiles) differ markedly from those of animals of the group Anamnia (amphibia and fishes). Of interest in this connection are the results of experiments with antigens from the crystalline lens of a fish (cod), since during interaction of these antigens with antisera to antigens from the lens of the chick in an agar gel, only slight clouding is produced. This shows that the crystalline lenses of fishes and birds evidently contain no common antigens, but only the common specific determinants of the group, which are responsible for the similarity in the antigenic properties of the crystalline lenses of these two far-removed classes of animals. In support of this are the results obtained by electrophoresis [23]. It may be thought that the presence of common group determinants in the protein molecules of the lenses of very distantly related animals (fishes and birds) is associated with the identical function of the crystalline lens — the transmission of light.

We were confronted with the problem of whether the crystalline lenses of vertebrates and invertabrates have any common antigenic properties. The eyes of the Cephalopoda are of greatest interest, for in their structure and function they are very similar to the eyes of vertebrates [2, 17, 18]. However the eyes of the cephalopod molluscs — and in particular the crystalline lens — have a completely different origin from those of vertebrates [3, 9, 19 and others], i.e. the eyes of the cephalopod molluscs and the vertebrates are analogous organs and afford a striking example of convergent homology. For this reason the comparative study of the antigenic properties of the crystalline lenses of the cephalopod molluscs and the vertebrates is of great interest.

The results obtained by means of the anaphylaxis reaction showed (Table 3) that all the guinea pigs sensitized to antigens of the crystalline lens of the chick, cod and calamary, responded to injection of saline extract of lens tissue of the cod by marked signs of anaphylactic shock. However, in response to injection of saline extract from lens tissue of the chick into guinea pigs sensitized with antigens from the crystalline lens of the calamary, we observed a feebly developed anaphylactic shock in one animal only and in the other two guinea pigs no signs of anaphylactic shock could be seen.

The absence of anaphylactic shock in the two guinea pigs is evidently due to the fact that the antigens of the calamary used for sensitization have very few determinant groups similar to those of the antigens used for the assaulting injection (saline extract of lens tissue of the chick). At the same time, injection of a saline extract of crystalline lens tissue of the chick into guinea pigs sensitized with antigens of the lens of the chick and cod, signs of anaphylactic shock were observed. After injection of saline extracts of the liver tissue of the cod and chick into guinea pigs, sensitized with antigens of the crystalline lens of the chick, cod and calamary, we observed no signs of anaphylactic shock. Injection of saline extracts of the crystalline lens tissue of the cod into nonsensitized guinea pigs also gave no signs of anaphylactic shock.

Thus the results obtained by the anaphylaxis reaction show that there is homology of the antigenic properties of the crystalline lenses of the cephalopod molluscs and the vertebrates and moreover that this homology is strong between the crystalline lens antigens of the cod and calamary, and almost imperceptoble between the lens antigens of very distantly related species — the chick and calamary. Hence it follows that the performance of identical functions by organs leads not only to homology of their structure but also to homology of their antigenic properties, in spite of their different origin. These findings are in agreement with the experimental results of I. I. Titova [8], who demonstrated the homology of the antigenic properties of the crystalline lens tissue of the triton, which is derived in embryogenesis from the ectoderm and the crystalline lens formed the iris during regeneration.

SUMMARY

The results of our experiments using the precipitation test in agar showed that the presence of common antigens in the crystalline lenses of various animals is associated not only with convergence, but also to a large degree, evidently, with the family relationships between the various species of animals. Hence, in the comparative study of the antigenic properties of organs in different animals it is essential to take into consideration the degree of relationship between the animals and the possibility of convergent homology of the organs resulting from performance of the same function.

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REESTABLISHMENT OF CELL DIVISION AFTER REACTIVE INHIBITION BY DEPRESSION OF THE SYMPATHETICOADRENAL SYSTEM

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The existence in the body of factors and systems regulating the processes of proliferation of cells in various tissues and organs has recently been proved by a number of special investigations, including some by us [1-8, 10]. It has been established that the widest variety of stimuli in the external environment, which produce excitation of the body and an increase in the tone of the nervous sytem, alter the intensity of proliferation of the tissue cells. Under these circumstances the mitotic activity usually falls; reactive inhibition of cell division is observed.

Friedenwald and Buschke [10], who first discovered the phenomenon of reactive inhibition of the processes of cell division, put forward the view that this reaction is effected through a humoral mechanism. The experimental observations of these workers, and the later research by Bullough [9], the author [4, 6], I. A. Alov [1] and others showed that an analagous depression of cell division is also observed after injection of adrenalin into the animal. On the other hand, it was shown in work by I. A. Alov [1] and A. K. Ryabukha [6], that reactive inhibition of cell division is not observed or is considerably weakened in adrenalectomized animals.

It may therefore be regarded as proved that the mechanism of reactive inhibition of the proliferation of tissue cells is a neurohumoral one, and that one of its main links is the adrenals.

Continuing the study of the laws of cell division and the relationship between the processes of division and various physiological conditions of the body, we undertook an investigation of the changes in cell division during the frequent and prolonged action of stimuli on the animal when the nervous system was either in a condition of excitation or of depression. The question arose, what takes place in these conditions in the processes of cell division; how long can reactive inhibition be maintained; do not the regulating mechanisms become exhausted, and if so, how is this reflected in cell division; do the cells themselves show adaptation to the humoral factor inhibiting division, and so on.

EXPERIMENTAL METHOD

We carried out experiments on white mice of the same age and sex. Prolonged stimulation of the animals was carried out by a weak electric current of the minimum voltage to which they reacted, in a special chamber with a metal floor which was included in the circuit. Stimulation was by means of isolated impulses of 3-4 sec duration, at intervals of 6-8 sec, for 4 hr 30 min, 7 and 9 hr. The circuit was opened and closed automatically.

In other experiments we used adrenalin as a long acting stimulus. The hormone was given as a 1:20,000 solution, in a dose of 0.2 ml per injection. In view of the instability of the preparation, it was injected repeatedly into the animals: each hour for 4 and 7 hours, every 2 hours for 10 hours, and every 3 hours for 24 hours.

Remembering the natural daily variations of mitotic activity, a separate control was provided for each experiment. The control animals were killed at the same times as the experimental animals. The test object was the stratified epithelium of the cornea of the eye. The material was fixed in Bouin's fluid. Total preparations of the cornea were obtained (stained by Caracci's method and by Yasvoin's iron-hematoxylin method); some of the material was embedded in paraffin wax for the preparation of histological sections (stained with hematoxylin-eosin).

To determine the mitotic activity, 100 fields were examined in each cornea from the animal and the topographical pattern of the mitoses was compared; we took into consideration both the total number of dividing cells and their phase relationships.

EXPERIMENTAL RESULTS

In our previous investigations [3, 6] we showed that transient, mechanical painful stimulation of the animal or the action of an electric current on the animal for 15 to 30 min leads to severe changes in the phase relationships of mitotic division; under these circumstances the early stages of mitosis as a rule disappear. A shift is observed in the phases also after action on the animal for 5 minutes, if the material is not fixed immediately but after a short time, e.g. 30-40 min.

Increase in the length of the time of action to 1 - 2 hours causes the reactive inhibition of cell division to extend to all the phases of mitosis. Stimulation for 60 minutes is usually enough to cause almost complete suppression of the processes of division. Mitotic activity falls by 91-93% by comparison with the controls, and only solitary dividing cells remain. Further increase in the period of action to 2-3 hours causes only little change in the reactive inhibition of cell division. The processes of cell proliferation are practically completely inhibited; nevertheless a very small percentage of mitoses remains even under these conditions.

The solitary dividing cells still do not disappear after stimulation for 4 hours; in fact in this case some increase in the number of mitoses may even be observed, mainly in the earlier stages of division — the prophase. This chance observation was confirmed by a special investigation. In all experiments without exception in which stimulation lasted 4 hr 30 min we observed a shift in the opposite direction in the phases of mitotic division.

As seen from Table 1, in which are shown the results of one of these experiments (animals were stimulated by an electric current for 4 hr 30 min), the mitotic activity was at the rate of 10% of the initial level, and the proportion of early stages of division was relatively high.

Ana-and telophases are almost absent; this suggests that division was completely suppressed not long before the end of the experiment. The number of prophases reaches 34% of the control figure, and this undoubtedly is an indication of the incipient increase in mitotic activity in the epithelium.

TABLE 1

Reestablishment of Mitotic Activity in Animals During Prolonged Stimulation

Duration of stimulation	Number of corneas	Phase of mitosis				Total number of mitosis
		P*	M	A	T	
Control	8	29	27	17	43	116
4 hr 30 min	6	10	1.5	0	0.7	12
%,control fig.	—	34	5	0	1.6	10
Control	6	20	30	18	39	106
7 hr	6	16	7	6	9	39
%, control fig.	—	80	23	33	23	37

*Here and in the following tables P, M, A and T indicate pro-, meta-, ana- and telophase.

TABLE 2

Mitotic Activity of the Corneal Epithelium After a Single Injection of Adrenalin to Animals Subjected to Prolonged Stimulation

Time elapsing after injection of adrenalin	Number of corneas	Phases of mitosis				Total number of mitosis
		П	М	А	Т	
Control	6	20	30	18	39	106
40 minutes	4	2	0	1	9	12
1 hr 30 min	6	2	0	0	1	3

TABLE 3

Suppression of the Restoration of Cell Division by Adrenalin on Its Re-establishment After Previous Inhibition

Duration of stimulation	Number of corneas	Phases of mitosis				Total number of mitosis
		P	M	A	T	
Control	6	15	15	10	24	64
9 hours	6	17	11	5	11	45
% control fig	—	—	—	—	—	70
9 hours + adrenalin	6	0	0	0	5	5

In another series stimulation of the mice continued for 7 hours. Under these conditions clear signs of exhaustion of the animals and depression of their nervous system developed, although the stimuation was applied in the form of separate short impulses with longer pauses in between. Table 1 shows that, in spite of the continuing action of the stimulus, usually leading to sharp suppression of cell division, the process of reestablishment of mitotic activity progressed further.

The number of mitoses in the corneas of the experimental animals amounted at this time to 37%, i. e. it was still considerably lowered. The intensity of division continued to rise slowly; in this case, however, there was a preponderance of prophases, the number of which reached 80% of the control figure. Similar results were also obtained in the other experiments.

From an analysis of our findings it can be deduced that the processes of proliferation of cells which are subjected to inhibition as a result of prolonged stimulation are reestablished despite the continuing stimulation. Restoration begins after approximately 4 hours; under these circumstances, we may believe, not all the "inhibited" cells begin to undergo division at the same time, otherwise the reestablishment of the initial level of mitotic activity would be observed one hour after the beginning of restoration, since the duration of mitotic division in the corneal epithelium is approximately 1 hours. In fact, as may be seen from the results shown, the process of restoration lasts for much longer. It is not impossible that under these circumstances the time taken for mitosis itself in the experimental animals should also be lengthened to some extent.

What is the cause of this restoration of the processes of proliferation of the cells? It may be due to two factors. First, it may be due to adaptation of the cells to the factor which prevents the transition to mitosis. The second possible cause is depression or exclusion of the neurohumoral mechanism of reactive inhibition of division.

In order to elucidate this problem we carried out the following experiments. Animals were exposed to stimulation for 6-7 hours, after which they were at once given an injection of adrenalin. Some of the mice stimulated for 6 hours were killed 40 minutes after the injection, while the animals of the other group were

stimulated for 7 hours and received two injections of the hormone: immediately after stimulation and after another 30 minutes; the material was fixed 1 hr 30 min after the first injection.

TABLE 4

Mitotic Activity of the Corneal Epithelium after Repeated Injection of the Animal with Adrenalin (5 injections) Over a Period of 4 hr 30 min

Observation	Number of corneas	Phases of mitosis				Total number of mitoses
		P	M	A	T	
Control	6	18	17	8	32	75
Experiment	6	0	0	0	0	0

As seen from Table 2, the mitotic activity in the corneal epithelium of the animals killed 40 minutes after injection of adrenalin is considerably reduced, and the total number of mitoses is one third that of animals exposed to the same stimulation (compare Table 1) without the subsequent injection of adrenalin. The early phases of mitosis are almost completely absent; the telophases are still comparatively numerous, since after 40 minutes they are unable to proceed into the resting stage. Such a relationship between the phases indicates that before injection of adrenalin the mitotic activity was at a very high level, i. e. reestablishment of the processes of division had taken place after their inhibition.

In the animals of the second group, killed 1 hour 30 minutes after the first injection of adrenalin, total suppression of division is observed.

Thus the processes of cell proliferation, reestablished during prolonged stimulation of the animal, may be suppressed secondarily by injection into the animal of the hormone of the adrenal medulla. It is, therefore, not a question of adaptation of the cells, nor yet that the cells cease to react to adrenalin, which is secreted in increased amounts during excitation of the animal. The cause of the reestablishment of cell division during prolonged stimulation of the animal is depression of the sympatheticoadrenalin system. In the conditions of our experiments the adrenals ceased to react with increased secretion of adrenalin, and the phenomenon of reactive inhibition of mitotic activity gradually disappeared. Continuing stimulation did not produce suppression of cell division, although the animals reacted as before to the stimulus. This conclusion is confirmed by the next experiments (Table 3). The phenomenon described may be regarded, it seems, as regulatory adaptation on the part of the animal.

We were interested to ascertain whether the mitotic activity is completely restored to its original level during continued stimulation. Animals were stimulated for 9 hours, and some of them killed immediately afterwards. From Table 3 it is seen that at this time a fairly large number of mitoses is observed in the corneas of the experimental animals; their total number still does not reach the control figure, but mitotic activity is already 70% of this value and it is clear that it will rise to the original level—this is shown by the phase relationships.

The animals of the second group, which were stimulated for the same length of time, were injected with adrenalin, and then killed 40 minutes later. As it was to be expected, secondary suppression of cell division was observed in the epithelium of these animals.

In connection with the results obtained, the question naturally arises whether restoration of the processes of division will be observed also after the prolonged injection of adrenalin into the animals; or whether, in spite of the possible depression of the nervous system under these conditions, cell division will be suppressed for a long time. Under these circumstances, what morphological changes may take place in the tissues?

In order to elucidate these problems we performed a series of experiments. Animals were injected subcutaneously with 0.2 ml of a 1:20,000 solution of adrenalin repeatedly over a long period of time. In Table 4 are shown the results of these observations. The hormone was injected 5 times in the course of 4 hours, i. e.

TABLE 5

Prolonged Inhibition of the Processes of Division by Repeated Injection of Adrenalin into the Animal

Duration of injection	Time of fixation after last injection	Number of corneas	Phases of mitosis				Total Number of mitosis
			П	М	А	Т	
For 7 hours (every hour)	Control	6	32	29	11	24	96
	After 45 minutes	6	1	0,5	0	0	2
	After 2 hours	6	4	2.5	1.5	1	9
For 10 hours (every 2 hours)	Control	6	43	63	26	39	171
	After 1 hour	4	2	3	1	0	6
	After 2 hours	4	6	7	5	8	26
For 24 hours (every 3 hours)	Control	4	24	25	11	27	87
	After 1 hour	2	2	1	0	1	4
	After 3 hours	4	37	22	19	23	101

once every hour. The material was fixed 30-45 min after the last injection. As can be seen, the mitotic activity was completly suppressed. There were no signs of restoration of division. It is interesting to recall that at this period of stimulation of the animals with the electric current, an obvious reverse shift was observed in the relationships of the phases of mitosis (see Table 1).

In another series of experiments adrenalin was injected over a period of 7 hours, also at intervals of 60 min (Table 5). When the material was fixed 45 min after the last injection, as in the previous case total suppression of division was observed. Some mice were killed 2 hr after the last injection. In the corneas of these animals an increase was noted in the number of early stages of division. At this time, therefore, the action of adrenalin has already come to an end.

As we know, adrenalin is an unstable compound; it is rapidly inactivated in the body as a result of enzymic oxidation. Because of this, we also injected animals with adrenalin over a longer period of time—10 hours (every 2 hours) and even for 24 hours. It should be mentioned that the mice tolerated the repeated injections of adrenalin very well indeed; none of the animals died as a result.

As may be seen from Table 5, as a result of injection of the animals with adrenalin the processes of cell division are not restored during the whole period of injection of the hormone, namely 10 hours. In the case of painful stimulation of the mice, at the 9th hr from the start of the experiment (see Table 3) a well marked increase in mitotic activity is observed, reaching 70% of the original level.

Tissue cells thus do not show adaptation to adrenalin, and even after repeated injections of this hormone they still retain the power to react with inhibition of division. In the last experiment (as in the previous one), when the material is fixed 2 hours after the last injection, an accumulation of mitoses is observed. Hence the state of inhibition of division lasts no longer than 2 hours after the injection of the hormone has come to an end. Investigation of the mitotic activity in the corneal epithelium of animals in which adrenalin was injected every 3 hr for 24 hr shows convincingly that (see Table 5) in this case cell division was suppressed. In mice killed after 1 hour, mitoses were almost absent. However, when the material was fixed 3 hr after the last injection, the number of dividing cells was slightly greater even than the number in the control animals (116%).

Transverse sections of one of the corneas of each animal in the last series (both experimental and control) were examined histologically. Under these conditions of activity on these animals, no essential morphological changes in the corneal tissues could be found. However, the possible suppression of cell division in the tissues for a long period of time, with consequent interference with the course of physiological regeneration during frequent or prolonged stimulation of the animal, gives grounds for the suggestion that morphological changes in the tissues and organs are also possible under these circumstances. This problem clearly has a bearing on practical medicine and calls for special study.

SUMMARY

In prolonged stimulation of mice by weak electric current the reactive inhibition of the mitotic activity in the corneal epithelium ceases. However, in 4 hours it reappears notwithstanding the continuing stimulation. Adrenalin administration again depresses the mitosis. If this hormone is administered every 2-3 hours it is possible to inhibit the tissue cells mitosis for hours (even as long as 24 hours). There was no adaptation of cells to adrenalin.

Thus the cause of the reestablishment of mitotic activity in prolonged action of the current consists in depression of the neurohumoral mechanism responsible for the inhibition of mitosis, mainly of adrenal system.

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THE MITOTIC ACTIVITY OF THE EPIDERMIS OF THE LIMB IN RATS AFTER DENERVATION

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Many investigations have shown that the nervous system plays a considerable part in the regulation of the proliferation of cells. Changes in mitotic activity have been demonstrated in experiments in which the innervation of tissues was destroyed [1, 3, 5, 6, 8]. The conflicting nature of the findings do not, however, permit any conclusions to be drawn on the character of the influence of the nervous system on cell division. We accordingly set out to study the changes in mitotic activity occuring during certain forms of interference with the peripheral nervous system.

In the present paper are described the results of observations made on the changes in mitotic activity in the epidermis after denervation of the limb.

EXPERIMENTAL METHOD

Experiments were carried out on sexually mature male white rats (average weight 180 g). The nerves of the hind limb of the experimental animals were divided on the left side: – the femoral nerve at the level of Poupart's ligament, the obturator nerve in the pelvis and the sciatic nerve immediately at its point of emergence from the pelvis.

In each case a portion of the nerve about 0.5 cm long was resected. All the operations were carried out under ether anesthesia. Sixty-one rats were used in the experiments.

On the 10th day after operation pieces of skin measuring 4 x 8 mm were excised from the upper third of the outer side in both limbs. At this time the operation wounds had completely healed. The material was embedded in celloidin paraffin. Sections were cut to a thickness of 7μ and stained with hematoxylin by Caracci's method.

The mitotic coefficient was calculated (the ratio between the number of dividing cells and the total number of cells, expressed in per thousand). In each case not less than 3000 cells were counted. In certain series we also determined the number of amitoses and measured the thickness of the epidermis. The counting of the cells and mitoses was done under a 90x objective and a 7x eyepiece. When comparing the mitotic coefficients and the other indices also, the significance of the random variation of their values – P – was determined by the Fisher-Student method. The differences were regarded as significant when P = 0.001.

EXPERIMENTAL RESULTS

In the 1st series (23 rats) we studied the changes in mitotic activity of the epidermal cells of the skin after section of the nerves of the limb as indicated above. The results obtained (see Table) show that the mean mitotic coefficient for the epidermis of the intact (not denervated) limbs (7.3 per 1000) was higher than that for the

TABLE

Mitotic Coefficient in the Epidermal Cells of the Denervated and Intact Limbs in Rats

Experimental animal number	Intact limb		Denervated limb		Changes in the mitotic coefficient of the denervated limb compared with the intact, in %
	Number of mitoses	mitotic coefficient (per 1000)	Number of mitoses	mitotic coefficient (per 1000)	
14	8	2.1	6	1.8	85.7
38	12	3.7	7	2.2	59.5
88	12	3.8	17	5.1	134.2
57	14	4.0	8	2.5	62.5
23	14	4.2	17	5.3	126.2
54	14	4.4	11	3.5	79.8
86	14	4.6	8	2.5	54.3
24	15	5.0	9	2.8	56.0
47	18	5.4	11	3.5	64.8
59	17	5.5	24	7.4	134.5
81	18	5.6	20	6.7	119.6
36	20	6.1	18	5.5	90.2
7	22	6.6	17	5.5	83.3
76	23	7.7	11	3.6	46.8
50	25	7.8	16	5.0	64.1
62	29	8.5	12	3.6	42.4
82	29	9.0	18	4.5	50.0
52	28	9.2	10	3.2	34.8
17	32	10.5	23	7.3	69.5
30	38	12.0	22	6.9	57.5
11	37	12.1	20	6.6	54.5
63	47	14.1	33	10.5	74.5
2	53	15.1	14	4,5	29.8
Average	23.4	7.3	15.3	4.8	65.7

epidermis of the denervated limbs (4.8 per 1000). This difference was statistically significant (P = 0.009).

A count of the number of mitoses by phases showed that the differences in the mean percentage values for each phase of mitoses in the epidermis of the comparable limbs were extremely insignificant.

The number of early prophases in the epidermis of the intact and denervated limbs, for instance, was respectively 25.4 and 29.5%, prophases – 31.9 and 28.7%, metaphases – 29.9 and 29.6%, anaphases 6.3 and 6.0% and telophases – 6.5 and 6.3%. At this period of time after operation, denervation of the limb thus has no essential effect on the rate of any of the phases of mitosis. This suggests that the reduction in the number of mitoses in the epidermis of the denervated limb is due to a reduction in the number of cells starting to undergo division.

The total number of amitotic figures in the epidermis of the denervated and intact limbs (3 and 14 amitoses respectively) was so small that it ruled out the possibility of making any conclusions about the influence of section of the nerves of the limb on the amitotic activity of the epidermis.

No essential differences were found in the thickness of the epidermis in the denervated and intact limbs.

In order to reveal any possible effect of trauma on the mitotic activity of the epidermis, we carried out a second series of experiments. The animals in this series were divided into two groups. In the first group of 10 rats the same operation of division of the nerves of the left limb was performed as in the animals in the 1st series of experiments; in the animals of the 2nd group (11 rats) the soft tissues of the left limb were incised and the

nerves left undamaged. The material in this case also was fixed on the 10th day after operation.

It was found that the animals in which only the soft tissues were incised showed a mean mitotic coefficient in the epidermis of both limbs which was almost the same: in the epidermis of the left limb, for instance, it was 12.1 per 1000 and in that of the right – 11.6 per 1000. In the first group of animals in this series of experiments, the mitotic coefficient in the epidermis of the denervated limbs was 7.7 per 1000, and in the epidermis of the intact limbs – 10.2 per 1000. This difference, like that in the 1st series of experiments, was statistically significant ($P = 0.003$). The higher value of the mitotic coefficient in the epidermis of those animals in which only the soft tissues were incised (12.1 per 1000) than that in the epidermis of the intact limbs of the experimental animals (10.2 per 1000) is not significant ($P = 0.105$).

Thus the results of the two series of experiments demonstrate a fall in the mitotic activity of the epidermis of the denervated limb. At the same time it is still not clear whether this fall is on account of a reduction in the number of dividing cells in the denervated limb, or of an increase in the mitotic activity of the epidermis of the opposite (intact) limb. It is true that a comparison of the mitotic coefficients of the epidermis of the undamaged limbs in the animals of the 2nd series of experiments (11.6 and 10.2 per 1000) shows no such increase. However the considerable individual variations in the mitotic activity of the animals prevents these findings from being regarded as sufficiently convincing. For this reason we attempted, in a third series of experiments, to elucidate this problem by comparing the mitotic activity in the epidermis of the fore-and hindlimbs of the same animal.

In the experimental animals of this series (12 rats) the left hindlimbs was denervated, and on the 10th day the mitotic activity was studied in the epidermis of all 4 limbs. Control animals (5 rats) were not subjected to any form of interference. In the control animals the number of mitoses in the epidermis of the fore-and hindlimbs was found to be almost the same.

The mean mitotic coefficient in the epidermis of the forelimb for instance, was 2.6 per 1000, and in the epidermis of the right hindlimb 2.1 per 1000 and the left hindlimb – 2.4 per 1000.*

The number of mitoses in the epidermis of both forelimbs and in the epidermis of the intact hindlimb of the operated animals also showed no significant differences. The mean mitotic coefficient in the epidermis of the right forelimb was 4.9, the left forelimb 5.4 per 1000 and the right hindlimb 5.3 per 1000. Meanwhile, in the epidermis of the denervated limb, the number of mitoses was considerably lower (2.9 per 1000). This fall in the number of mitoses is significant by comparison with their number in the right hindlimb ($P = 0.003$), the right forelimb ($P = 0.01$) and the left forelimb ($P = 0.004$).

SUMMARY

Unilateral section of the nerves of the hindlimb carried out under the above mentioned experimental conditions, does not, at a given period of observation, lead to any form of change in the mitotic activity of the epidermis in the contralateral limb; this does not, however, rule out the possibility of such changes as have been shown by several workers in experiments performed under different conditions [2, 4, 6, 7]. The conclusion may be drawn from the results of all 3 series of experiments that interruption of the innervation reduces the mitotic activity in the epidermis of the denervated limb.

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A STUDY OF THE EFFECT OF NOVOCAIN BLOCKADE ON INTACT MUSCLE BY MEANS OF VITAL STAINING

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Lumbar paravertebral block with novocain is widely used in medical practice. The nature of the effect of this block on the muscles has not, however, been adequately explained. Striated muscles must be the most reactive, for their functional activity is under the constant influence of the nervous system. The effect of the this is shown, as was pointed out by I. S. Beritov [2], by the high excitation of the denervated muscle in the region of the nerve endings. Novocain acts on the different organs through the means of nerve conductors. I. N. Kantorovich pointed out [7], for instance, that novocain causes a sharp reduction in the functional lability of muscle, and direct stimulation of the muscle produces a normal contraction even when the action of novocain is far advanced. L. G. Vol'fenzon [4] studied the action of novocain on the cells of different tissues, especially muscles, using the method of vital staining. This worker showed that novocain, in doses causing necrosis, produces a contraction of the rectus abdominis muscle of the frog, but this effect is not observed on the sartorius muscle.

Our object was to discover the effect of lumbar novocain block on the skeletal muscles of the hindlimbs in mammals.

EXPERIMENTAL METHOD

Experiments were carried out on white mice of both sexes, weighing on the average 20-22 g. We used the method of vital staining with neutral red. The method is described in detail in our previous paper [5]. An injection of 0.2 cm^3 of a 0.25% solution of novocain was given into the left sympathetic trunk in its lumbar division. To test the specificity of the action of novocain block, another group of animals was injected with the same volume of physiological saline. In response to a single injection of adrenalin we studied the sorptive power on the day of injection and the following 3 days; if the injections were given 2 and 4 times in the course of 2 days, the sorptive power was determined during the next 2 days; when four daily injections were given the mice were taken for study of the sorptive power on the last day of the injections. Altogether 88 mice were used in the experiments.

EXPERIMENTAL RESULTS

No fundamental differences were found in the reaction of the mice to injection of novocain or physiological saline into the sympathetic trunk. How may the fact that all the reactions were of the same type be explained? The workers who introduced the method of novocain block [3] emphasized the nonspecific nature of its action. In this connection attention is drawn to the findings of G. A. Ionkin [6], who demonstrated the increased lability of cardiovascular tone as a result of novocain block, and showed that similar changes appeared also after injection of hypertonic saline or of physiological saline. The common feature in the action of these solutions, in this worker's opinion, is the mechanical pressure of the injected fluid on the tissue and the change in osmotic pressure. I. N. Bel'gova [1] gave repeated injections of novocain and observed a considerable increase in the sensitivity of the heart. Prolonged action of Ringer's solution on the heart also increased its sensitivity to novocain.

TABLE 1

Values of the Sorptive Power of Muscles After Injection into the Sympathetic Trunk of Novocain Solution and of Physiological Saline, Expressed as Percent of Controls Which are Taken as 100% (experiments on females)*

Date	Control	Novocain (right limb)	Novocain (left limb)	Physiological saline (right limb)	Physiological saline (left limb)	Notes
1955	A. After a single injection					
6/IX	100	71	67	53	67	Injections 5/IX
7/IX	100	67	59	125	111	on —
8/IX	100	77	77	83	71	—
19/IX	100	77	71	—	—	Sorption studied on the 4th day after injection of novocain
	100	62	62	—	—	
26/X	100	91	91	—	—	Sorption studied on the day of injection
	100	83	83	—	—	
	B. After two injections at 48 hr interval					
9/IX	100	77	67	90	83	Injections given on 5/IX
	100	90	90	67	67	8/IX
	100	77	77	100	83	—
10/IX	100	83	71	90	100	—
	C. After 4 daily injections					
28/X	100	77	86	83	91	Injections given on 25/X, 26/X, 27/X, 28/X
	100	71	77	53	55	
	D. After four injections at 48 hr intervals					
26/IX	100	83	83	71	71	Injections given on 23/IX, 24/IX, 25/IX & 26/IX
27/IX	100	59	59	59	67	

*Sorption of the right calf muscles was compared with that in the right calf muscles of control animals, and sorption of the left with that in their left calf muscles. The values for the control animals were taken as 100%.

In our experiments physiological saline is a weak stimulating agent. Injection of a volume of 0.2 cm^3 of this solution cannot be regarded as indifferent for so small an animal as a mouse. It must be pointed out that although the left sympathetic trunk was stimulated, corresponding changes were observed in both right and left muscles. This may possibly be explained by transmission of the stimulus from the sympathetic ganglia of the left trunk through the central nervous system to the ganglia of the right sympathetic trunk. The connection between the sympathetic ganglia and the central nervous system through receptors, particularly via the peripheral processes of the neurones of the intervertebral ganglia, was demonstrated by E. S. Levitskaya [9].

Our observations revealed essential differences in the reaction of the calf muscles to stimulation of the sympathetic trunk as observed in females and males. As seen from Table 1, in females a fall in the sorptive

TABLE 2

Values of the Sorptive Power of Muscles After Injection into the Sympathetic Trunk of Novocain Solution and of Physiological Saline, Expressed as Percent of Controls Which are Taken as 100% (experiments on males)*

Date	Control	Novocain (right limb)	Novocain (left limb)	Physiological saline (right limb)	Physiological saline (left limb)	Notes
1955	A. After a single injection					
22/IX	100	91	83	—	—	Sorption studied on the day of injection of novocain solution
	100	71	83	—	—	
26/X	100	91	100	—	—	
	100	57	57	—	—	
6/IX	100	182	200	167		Injection on 5/IX
7/IX	100	167	182	143	143	
8/IX	100	100	133	100	100	
	B. After two injections at 48 hr interval					
29/X	100	100	125	83	100	Injection given on 5/X & 8/X
	100	91	125	91	100	
	C. After 4 daily injections					
28/X	100	77	100	77	91	Injection given on 25/X (X, 27/X & 28/X
	100	91	100	71	91	
	D. After four injections at 48 hr intervals					
16/X	100	143	143	154		Injection given on 6/X, 9/X, 12/X & 15/X
	100	125	125	15		
17/X	100	143	143	133	167	

*Sorption of the right calf muscles was compared with that in the right calf muscles of control animals, and sorption of the left with that in their left calf muscles. The values for the control animals were taken as 100%.

power of the calf muscles was observed in all cases.

In males the sorptive power fell immediately after the injection; after a few hours and for the next 3 days increased sorption over that of the controls was observed (Table 2, section A). A reduced sorptive power was found after four daily injections and, in isolated cases, after two injections in 48 hours (see Table 2, sections B and C). After 4 injections at intervals of 48 hours, an increase of sorption was observed in all cases (see Table 2, section D).

Some figures, reflecting changes in sorption as a result of the block, are only insignificant variations from the controls. Within these limits variations are observed in the sorptive power of muscles in the control animals (of the order of 10%). These cannot be regarded as variations in the sorption of muscles of different animals, since a definite tendency is observed in the changes in sorptive power of the experimental animals' muscles towards either a decrease or an increase of sorption.

Thus in the first place we succeeded in revealing sexual differences in the reactions of the calf muscles during lumbar novocain block. In the majority of males we observed an increase in the sorptive power of the

muscles during the action of the block. In the first few hours after the block we observed that the sorptive power was lower than in the control; on the next day we found an increase of sorption, which lasted until the 3rd or 4th day after the injection. In some cases sorption increased after multiple injections and in others it decreased. In females we observed only one type of reaction, namely a decrease in sorption. Hence a phasic type of reaction is observed in the muscles of the males: a phase of decreased sorption alternates with a phase of increased sorption.

The phasic nature of the response of living matter to an external agency was pointed out by S. N. Romanov [10, 11]. Sexual variations in the reaction of the epithelium of Descemet's membrane in rodents to strychnine were discovered by V. L. Levin [8], who also used the method of vital staining as a criterion of the sensitivity of the epithelium. Tissue from males not only showed itself to be more susceptible to injury by large doses, but also responded more rapidly to the action of minimal concentrations of strychnine.

The fact that in females only a phase of decreased sorption was observed in response to all the variants of the injection technique used, in contrast to the character of the reactions of the muscles in males, may be evidence of a considerably smaller degree of lability of the muscles in females.

SUMMARY

The sorptive properties of gastrocnemius was studied in mice (by vital staining with neutral red) in injection of 0.2 cc of 0.25% novocain solution into the lumbar portion of the left sympathetic trunk.

A decrease of the sorptive function of the muscles was observed in females. In males, after a short decrease of the sorption, this function was intensified on the next day after the injection. This increase was still present on the 3rd and the 4th day.

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MORPHOLOGY AND PATHOMORPHOLOGY

MORPHOLOGICAL CHANGES IN THE HEART, LUNGS AND BRAIN AFTER EXPERIMENTAL CAVOPULMONARY ANASTOMOSIS

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Vascular anastomoses (the subclaviopulmonary of Blalock, the aortopulmonary of Potts and the cavopulmonary of N. K. Galankin) are the most effective and easiest methods of treatment of a number of congenital heart lesions and are being used on an ever increasing scale in the clinics of the Soviet Union.

Of particular value for the clinician is the cavopulmonary anastomosis, which was suggested and developed in the A. V. Vishnevskii Institute of Surgery of the AMN SSSR.

The technique of the operation is as follows. A right-sided thoracotomy is performed with resection of the third rib. The superior vena cava and the right branch of the pulmonary artery are isolated. The latter is ligated at the point of its emergence from the pulmonary trunk, and is divided distally to the ligature. The superior vena cava is ligated distally to the azygos vein and is also divided distally to the ligature. The isolated peripheral ends of the superior vena cava and of the right pulmonary artery are joined to each other end-to-end by means of a Donetskii metal ring. As a result the flow of blood from the superior vena cava is shunted directly into the right pulmonary artery, by-passing the right atrium and right ventricle. In Fallot's tetralogy, because of the constricted outlet of the right ventricle, the volume of blood entering the lesser circulation is inadequate. The formation of a cavopulmonary anastomosis leads to an increase in the minute volume of the lesser circulation on account of blood entering from the superior vena cava.

The use of this operation in the clinic has yielded good immediate results (E. N. Meshalkin, A. A. Vishnevskii and N. K. Galankin). However there are as yet no clear ideas on the morphological changes arising in the body as a result of the formation of a cavopulmonary anastomosis.

The performance of the operation of cavopulmonary anastomosis is accompanied by a number of powerful and unaccustomed influences on the body – temporary clamping of the superior vena cava and shunting of the right pulmonary artery. As a result of the operation a considerable redistribution of the blood takes place, in consequence of which the filling of the right ventricle with blood is reduced by approximately 40% and the blood flow in the vessels of the right lung takes place at a much less pressure than normally. The aim of the investigation was to study the morphological changes in the brain, lungs and heart after the formation of a cavopulmonary anastomosis.

EXPERIMENTAL METHOD

Experiments were carried out on 21 adult dogs. After the formation of a cavopulmonary anastomosis they lived for periods from 3 hours to 5 months. An end-to-end anastomosis was formed between the peripheral ends of the superior vena cava and the right pulmonary artery in 13 dogs and an end-to-side anastomosis in 8 dogs.

TABLE

Summarized Results of the Experiments

Experiment No.	Type of anastomosis	Postoperative complications	Thrombosis of the anastomosis	Duration of survival after operation	Cause of death of the experimental animal
9	End-to-end	—	None	15 hours	killed
11	The same	Pleurisy and pneumonia	»	22 days	Pleurisy
12	» »	Pneumonia	»	8 days	Pneumonia
13	» »	Bilateral pneumothorax	»	3 hours	Bilateral pneumothorax
20	» »	Pleurisy	»	18 days	Killed
21	» »	»	»	75 »	Pleurisy
22	» »	»	»	5 »	»
23	» »	»	»	30 »	»
26	» »	Bilateral pneumothorax	»	3 hours	Bilateral pneumonia
27	» »	No complications	»		Killed at the end of the operation
29	» »	The same	»	3 days	Cause uncertain
30	» »	» »	»	5 months	Died from strangulation by the chain
32	» »	» »	»	36 days	Killed
34 38	Pleurisy, pericarditis	» »	»	24 hours	Died from strangulation by the chain
	The same	Pleurisy, pericarditis	Mural thrombus	14 days	Thrombosis of the right atrium
40	» »	Bilateral pneumothorax	None	20 hours	Bilateral pneumothorax
41	» »	Pleurisy	Thrombosis of the right atrium	15 days	Pleurisy
42	» »	Pleurisy, pericarditis	None	28 »	Bilateral suppurative pleurisy
43	» »	No complications	»	10 hours	Killed
45	» »	The same	Mural thrombus of the anastomosis	35 hours	»
46	» »	Pleurisy	Mural thrombus »	10 days	Pleurisy

At the time of formation of the end-to-end anastomosis the blood flow through the superior vena cava had to be completely interrupted for 30-40 minutes. When the anastomosis was formed end-to-side the superior vena cava was only partially occluded, so that the natural flow of blood along it was preserved. Only after the anastomosis was operating was the superior vena cava ligated proximally to the anastomosis, thereby directing the flow of blood from the superior vena cava to the pulmonary artery.

EXPERIMENTAL RESULTS

In the Table we give the summarized results of the experiments together with an indication of the complications and causes of death of the animals. As may be seen from these results, 8 dogs survived for up to 35 hours after the formation of the anastomosis (1st group), 6 dogs survived for 3 to 15 days (2nd group) and 7 dogs for 18 to 150 days (3rd group).

Histological examination of the heart, lungs and brain of the experimental animals was carried out. Fragments were excised from all parts of the lungs. All the divisions of the heart were examined: the anterior and posterior walls of the right and left ventricles, the interventricular septum, the right and left atria and the auricles of the right and left atria. For the study of the brain fragments were excised from its various divisions, enabling the cortex, basal ganglia and brain stem to be examined. The material was fixed in 10% neutral formalin solution. Sections were stained with hematoxylin-eosin, Scharlach red and by Nissl's method.

On histological examination of the lungs, irrespective of the length of time after operation and the method of formation of the anastomosis, well marked hyperemia was found. Vessels of all calibers were dilated and distended with blood. At early periods after operation (up to 28-36 days) foci of edema of the lungs were found on the side of anastomosis. Here and there the edema was more pronounced beneath the pleura than in the other parts of the lungs. Large foci of atelectasis and areas of emphysema were seen. Very large areas of pulmonary edema, with ruptute of alveolar septa were found in association with the use of artificial respiration under increased pressure. In places whole "lakes" were formed as a result of rupture of the alveolar septa by edema fluid. In the small and large bronchi edema fluid was also present. In 7 animals a fibrinopurulent pleurisy developed, in places with signs of organization of the exudate. In these places the pleura was greatly thickened with calcareous deposits. Here and there in the lung tissue were seen large and confluent foci of hemorrhage. In some preparations the alveoli were continuously bathed in blood and distended with red blood cells.

In one dog a thrombus with commencing organization was found in the main pulmonary trunk. A noteworthy feature was the almost complete absence of shrinkage of the lung or signs of sclerosis and atrophy of the lung parenchyma even at late periods after operation.

On examination of the heart, in 4 dogs fibrinopurulent pericarditis was found, in places with organization of the exudate. The epicardium in these cases was infiltrated with segmented leucocytes, eosinophils and plasma cells. In these animals foci of deposition of tiny droplets or clouds of fat were seen in the cardiac muscle fibers in various regions of the heart, most commonly beneath the epicardium. No other changes were found in the heart after careful histological examination.

On histological examination of the brain well marked hyperemia of the vessels was found. In 3 dogs multiple or solitary foci of hemorrhage had appeared in the brain substance (in two of these dogs the cavopulmonary anastomosis was formed end-to-end and in one, end-to-side). At late periods after operation no hyperemia of the cerebral vessels was found.

As a result of the anastomosis between the superior vena cava and the pulmonary artery changes develop in the body soon after the operation – hyperemia of the vessels of the lesser circulation with the appearance of foci of hemorrhage and edema in the lungs and foci of hemorrhage in the substance of the brain.

The fibrinous pleurisy and pericarditis develop in consequence of the opening of the pleural and pericardial cavities and are not directly concerned with the operation of cavopulmonary anastomosis; they are complications of intrathoracic and intrapericardial procedures in general.

According to S. M. Smirnov, ligation of a main branch of the pulmonary artery is followed by cirrhosis of the lung, which shrinks to $^2/_3$ of its original volume. The operation of cavopulmonary anastomosis is also attended by ligation of a main branch of the right pulmonary artery. However, in contrast to simple ligation of the artery, in cavopulmonary anastomosis the flow of blood from the superior vena cava is directed into the peripheral end of the right pulmonary artery. Evidently as a result of this, pneumosclerosis does not develop. The absence of pneumosclerosis after the operation of cavopulmonary anastomosis, despite ligation of the pulmonary artery, is one of the advantages of this operation.

A serious complication of the operation of cavopulmonary anastomosis is the appearance of foci of hemorrhage in the brain substance. As a result of the prolonged clamping of the superior vena cava and the consequent sharp rise in pressure therein, foci of hemorrhage develop in the brain, morphologically similar to those produced by diapedesis. Hence the severe and acutely developing venous hyperemia of the brain, resulting from the prolonged clamping of the superior vena cava and the associated increase in pressure in that system may cause in some animals the development of hemorrhages in the brain substance by diapedesis. The prolonged clamping of the superior vena cava during the operation of cavopulmonary anastomosis also causes the development of multiple foci of hemorrhage in the skin of the head, face, neck and shoulder girdle, as shown by examination of the cadaver of a patient who died soon after the operation.

The mechanism of development and the causes of the appearance of foci of hemorrhage in the skin and the brain are the same. For this reason the appearance of foci of hemorrhage in the skin must be regarded by the doctor in charge of the patient during the postoperative period as a serious sign of the possible development of hemorrhages by diapedesis in the substance of the brain.

This question of the prevention of foci of hemorrhage in the brain after the operation of cavopulmonary anastomosis is of real and urgent importance in the clinic, as is confirmed by our morphological investigations.

Occlusion of the superior vena cava is accompanied by a reduction in the minute volume of the right heart by approximately 40%. The sharp reduction in the blood flow must apparently cause changes in the heart as a result of the rapid change in the usual hemodynamic conditions. Nevertheless neither dystrophic nor atrophic changes were found on careful histological examination of all the divisions of the heart, which is another advantage of the operation of cavopulmonary anastomosis.

The complications of cavopulmonary anastomosis are in part a matter of the great, important and as yet incompletely solved problem of operative trauma.

To reduce the risks of the operation of cavopulmonary anastomosis it is essential to improve the operative technique. In the first place ways must be found of preventing the complications arising during occlusion of the superior vena cava.

SUMMARY

The authors studied the morphological changes occurring in the heart, lungs and brain after placing the cavopulmonary anastomosis. Experiments performed on 21 dogs established that prolonged occlusion of the superior vena cava during placing the cavopulmonary anastomosis may cause the development of diapedetic hemorrhages in the brain substance. The placing of cavopulmonary anastomosis may cause the development of fibrinous pleurisy and pericarditis. Cavopulmonary anastomosis is not connected with the development of pneumosclerosis, notwithstanding the ligature of the right pulmonary artery. No morphological changes are caused in the heart as a result of disengaging the superior vena cava from the right auricle. It is necessary to improve the technique of this surgical procedure to minimize its danger. It is of importance to reduce the time of occlusion of the superior vena cava during the operation.

CHANGES IN THE GLYCOGEN CONTENT OF THE NERVOUS SYSTEM AND MUSCLES IN ANIMALS INFECTED BY VIBRION SEPTIQUE

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We know that in muscle infected with gas hangrene there is a decrease in the amount of glycogen decomposed to form lactic acid, as a result of which acidosis develops in the tissues [1, 2]. It has been shown histochemically that the muscle fibers in the infected focus are lacking glycogen [7]. The content and distribution of glycogen in the various divisions and the various cells of the nervous system in gas gangrene have not been studied. There are grounds for the belief that glycogen has an essential influence on the activity of the nervous system, and evidently takes part in the restitution of the nerve cell and the regeneration of Nissl's granules [6]. Along with the gross changes undergone by the nervous system in gas gangrene [3, 4, 8], the degree of accumulation and distribution of glycogen in the nervous system may evidently be an indication of its condition.

These considerations provided a motive for the histochemical investigation of the muscles and the various divisions of the nervous system in white rats infected with vibrion septique in various experimental conditions that could have a bearing on the outcome of the infection.

EXPERIMENTAL METHOD

Observations were made on 4 groups of white rats (with 10 animals in each group), all of which were infected at the same time. They were each given an injection of one MLD of vibrion septique, mixed with 2.5% calcium chloride solution, into the calf muscle. Into the same muscle, either before or after infection, was injected 0.1 ml of a 0.25% solution of a long-acting preparation of novocain in apricot oil, or the same volume of sterile apricot oil alone. The animals of group I were injected with novocain 24 hours before infection, those of group II – 1 hour after infection, the group III animals were injected with apricot oil 24 hours before infection and the animals in group IV were infected without any further treatment (controls)*

From 4 rats of group I, 4 rats of group II, 3 rats of group III and 3 rats of group IV (killed 1, 7 and 45 days after infection), both calf muscles, both sciatic nerves, both S_1 spinal ganglia, the spinal cord, the medulla and the anterior lobes of both cerebral hemispheres were removed for histochemical investigation of the glycogen by the method of A. L. Shabadash. The presence of glycogen was demonstrated by treatment with diastase.

EXPERIMENTAL RESULTS

Complete loss of glycogen was observed in the muscle fibers of both calf muscles 24 hours after infection of the control rats. Glycogen was found only in the cells of the infiltrates and of the connective tissue on the right, and in the form of occasional granules in the endomysium on the left. In the rats of groups I, II and III

*Infection of the animals was carried out by Candidate Med. Sci. Z. I. Sobieva.

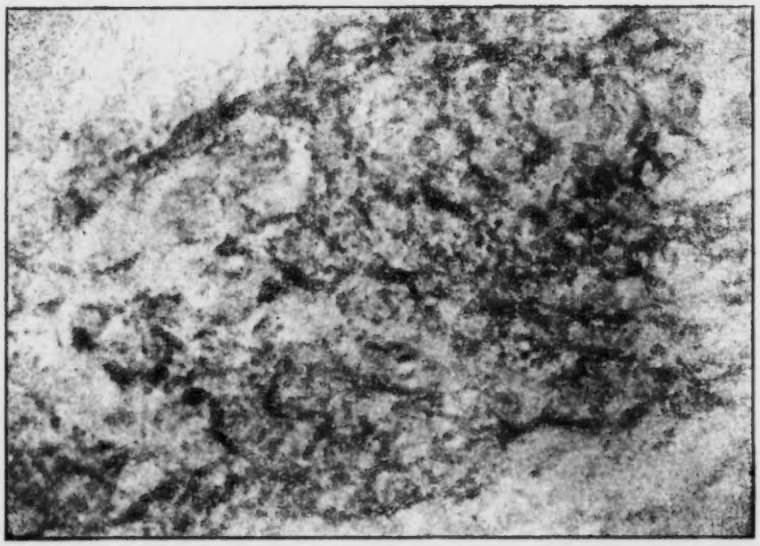

Fig. 1. Glycogen in the ependyma and in the wall of the lateral ventricle of the right cerebral hemisphere in a rat of group II on the 7th day after infection. (Shabadash method. Microphotograph 798 x).

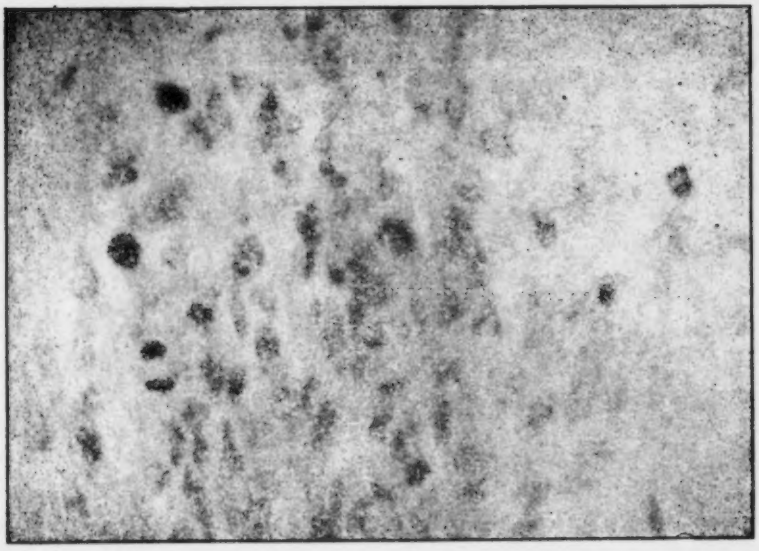

Fig. 2. Mitoses in the ependyma and in the wall of the lateral ventricle of the left cerebral hemisphere of a rat of group III on the 7th day after infection. (Shabadash method. Microphotograph 798 x).

this loss of glycogen by the muscle fibers was not observed. In the rats of group I glycogen was found in both muscles in the dark Q disks; in addition it was observed in the endomysium and the vessel walls. In the rats of group II, on the right there was no glycogen in the muscle fibers; it was seen in the form of isolated granules in the endomysium, the vessel walls and the cells of the infiltrates; on the left it was present in a few muscle fibers and also in the endomysium and vessel walls. In the rats of group III, glycogen was found on the right in the dark Q disks, the sarcoplasm, the endomysium and the cells of the infiltrates in a larger quantity than in the

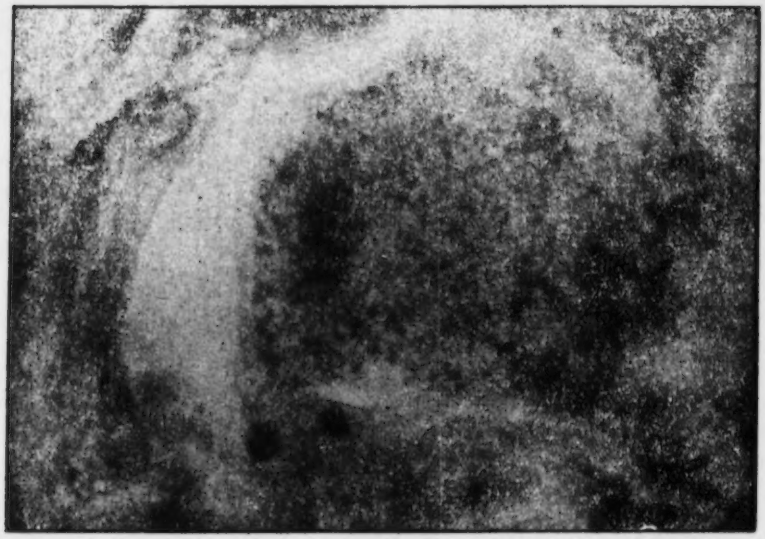

Fig. 3. Mitosis in the capsule of a nerve cell of the right spinal ganglion in a group II rat on the 45th day after infection (Shabadash method. Microphotograph 1260x.

previous rats; on the left there was less of it than on the right. Restoration of the normal content and distribution of glycogen in the various histological structures of the muscle tissue took place more completely in the rats given additional treatment than in the controls.

On the 7th day after infection glycogen was found in both muscles of a control rat in the dark Q disks of a few fibers; on the 45th day after infection it was also found in a few muscle fibers and in the endomysium.

In the rats of groups I, II and III on the 7th and 45th days after infection the glycogen content of the muscle on the right was higher than that of the control rats; sometimes it was higher on the right than on the left. Glycogen was found in the dark Q disks of many muscle fibers, and sometimes in the sarcoplasm and endomysium and also in the histiocytes and lipoid cells.

Naked eye and histopathological examination of the right and left calf muscles [5] showed that the course of the pathological process was most favorable and accompanied by intensification of proliferation of certain connective tissue cells and the appearance of mitoses therein, in animals receiving injections of oil, followed by those receiving an injection of novocain 24 hours before infection, then by those injected 1 hour after infection, and it was least favorable in the control animals (group IV).

Thus the content and distribution of glycogen in the muscles of the various groups of animals corresponds to our findings by histopathological examination of these animals: the course of the process is most favorable in the rats of group III, then in groups I and II, and least favorable in the control rats.

In the sciatic nerves and spinal ganglia glycogen was found only inside the lumen of vessels, in the cells of the blood or polyblasts. In the central nervous system glycogen was seen in the ependymal cells, in the walls and lumen of vessels, in the leucocytes and, rarely, in the glial cells. Its accumulation in the central nervous system was greater in animals injected with oil and novocain than in the controls, infected without any addtional treatment. This difference showed up most clearly on the 7th day after infection, and the highest glycogen content was found in the ependyma and wall of the lateral ventricles of the cerebral hemispheres in animals receiving novocain. In the rats of group I killed on the 7th day after infection, for instance, considerable deposits of glycogen were discovered in the ependyma and wall of the lateral ventricles. Still more glycogen was found here in the rats of group II, also killed on the 7th day (Fig. 1). In a control rat killed at the same time as these, there was a small quantity of glycogen in the ependyma of the left lateral ventricle, but none in the ependyma of the right ventricle. This difference is shown less clearly in animals killed on the 45th day after infection, for

in rats of groups I and II there was less glycogen in the ependyma of the lateral ventricles than in the rats of the same groups on the 7th day after infection, and glycogen was also found in the control rat, but in the form of solitary granules.

One more feature deserves attention. The number of cells undergoing mitotic division was higher in the various phases of division than normal in the ependyma and the wall of the lateral ventricles of the brain in the rats of groups I and III on the 7th day and in all the rats on the 45th day after infection (Fig. 2). Sometimes mitoses were found more often in the left hemisphere than in the right, i. e. in the hemisphere innervating the infected limb. Under these circumstances there appears to be a definite connection between the number of mitoses and the glycogen content. In a hemisphere with a high glycogen content there are few mitoses, and conversely, it is low where mitoses are common. In a group II rat killed on the 45th day after infection, we also observed mitosis in the capsule of a nerve cell in the right spinal ganglion (Fig. 3).

From information in the literature [9], in all vertebrates the ependyma and its derivatives are neurosecretory organs. Their neurosecretion is a complex of mucopolysaccharides and proteins. This secretion of the ependymal cells is transported from the basal area of the cells into the vessels, and from the apical area into the cerebrospinal fluid. In all probability the glycogen too, which we found in the ependyma of the lateral ventricles, is partially secreted or transported by the ependymal cells into the ventricular system, thereby demonstrating the possible participation of the cerebrospinal fluid in the pathological process.

The increased accumulation of glycogen in the ependyma and wall of the lateral ventricles of the cerebral hemispheres may be evidence of increased synthesis of glycogen or of its inadequate utilization on account of the lowered metabolic activity of the nerves when the animal emerges from a pathological state. If we bear in mind that the increased accumulation of glycogen in the wall of the lateral ventricles is expressed to the highest degree in the animals receiving injections of novocain and oil, and in which the pathological process followed a more favorable course than in the controls, then it must be concluded, it seems to us, that increased synthesis of glycogen is taking place here in association with the improved condition of the infected animal, as a phenomenon of a compensatory nature. Under these circumstances, the greater the mobilization of energy resources which arises, probably, to provide for the demands of the metabolism of the nerves, the more severe the course of the pathological condition. This is illustrated by the fact that in the rats injected with novocain one hour after infection, the accumulation of glycogen observed in the recovery period in the ependyma and wall of the lateral ventricles was greater than that in the animals receiving novocain and oil 24 hours before infection; the course of the pathological lesion was more severe in the former than in the latter.

The diminution which we observed in the accumulation of glycogen in the walls of the lateral ventricles during intensification of mitosis here, was presumably evidence of utilization of this resource of energy by the increased mitotic activity. In our view, the latter was probably connected with intensification of the compensatory reactions of the body. Hence in this case also, the glycogen content of the central nervous system evidently reflects the degree of compensation by the body of the disturbances resulting from the pathological process.

SUMMARY

A culture of vibrion septique was injected into the gastrocnemius muscle of white rats. A stable preparation of novocain on apricot oil, or apricot oil alone were injected into the same muscle.

Control animals were infected without any additional interventions. Glycogen was examined by A. L. Shabadash's histochemical method. A more favourable outcome of the infection was noted in the experimental animals in comparison with control. The loss of glycogen by the muscles during the most acute stage of the pathological process (24 hours after the infection) was less intense in experimental animals. The accumulation was also more intense in the muscles and in the central nervous system, especially in the ependyma of the lateral ventricles (in 7 days after the infection) in experimental animals. In reconvalescing experimental and control animals there were more mitotic figures in the wall of the lateral ventricles than in normal condition.

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METHODS

CONTINUOUS RECORDING OF THE SYSTOLIC BLOOD PRESSURE BY A BLOODLESS METHOD USING A SIMPLE TRACING SYSTEM

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Existing methods of bloodless recording of the blood pressure are less accurate than the direct method, but their use in physiological and clinical investigations is perfectly justified if the aim is simply to determine whether the pressure is raised or lowered, or to determine its relative value in cases where it is desirable to avoid operative procedures.

Among the many indirect methods of recording the blood pressure, one which deserves our attention is a method in which the air pressure in a sphygmomanometer cuff is maintained automatically at the level of the systolic (or diastolic) blood pressure by means of a mechanism controlled by the pulse waves. In recording the systolic pressure, air enters the sphygmomanometer cuff through an electromagnetic valve which opens only at the moment of passage of the pulse wave to the peripheral part of the limb. If the initial pressure in the cuff is at a lower level than the systolic blood pressure, with each pulse wave passing the cuff to the periphery a further quantity of air enters the system. The inflation of the cuff proceeds until its pressure is no longer below the systolic pressure. At this moment the pulse wave at the periphery disappears and the valve closes. In consequence of a controlled leakage of air from the system (or by means of the automatic opening of a special escape valve) the pressure inside the system begins to fall until the pulse reappears. The first pulse wave to reach the periphery opens the inlet valve, and the whole cycle is repeated once more. Thus the pressure inside the cuff follows the changes in the systolic pressure, sometimes rising above its level and at others falling below it.

The various modifications of this method are mainly concerned only with the method of detection of the pulse wave at the periphery of the limb. For this purpose additional cuffs [1, 4, 5, 6, 8], a pneumatic finger plethysmograph [2], a photoelectric finger plethysmograph [7] and a piezoelectric detector [3] have been used. In spite of the comparatively large number of schemes suggested, the method just described has not been widely used in practice, in view of the relative complexity of the apparatus and of a number of general disadvantages which reduce the accuracy of recording. The most important of these are: the fact that the working of the electromagnetic valve depends on volume changes in the peripheral part of the limb, unconnected with changes in pressure; the considerable overdistenion of the veins with blood; distortion of the recording by movements of the muscles and the high inertia of the system.

The system which we propose, which is suitable for investigations on man and for chronic experiments with dogs, differs slightly in its principle of action from the existing apparatus, and is free from certain of the disadvantages which have been mentioned. The main difference is that the working of the electromagnetic valve is controlled by oscillations of pulse pressure in the sphygmomanometer cuff itself, and not by pulse waves in the peripheral parts of the limb. This prevents distortion of the results from local vasomotor reactions and from passive changes in the filling of the vessels with blood, since the entire network of vessels smaller than the main

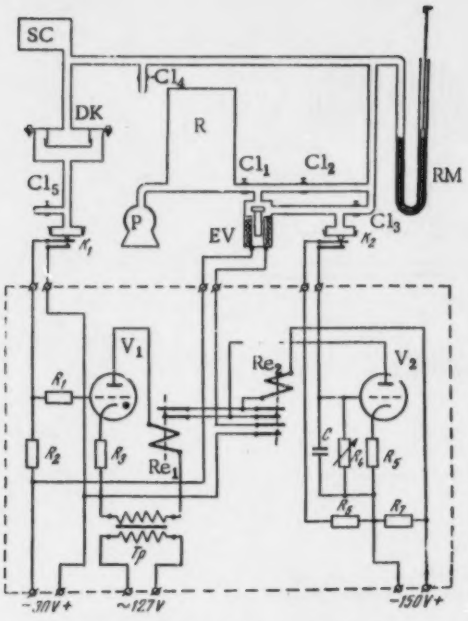

Fig. 1. Essential scheme of the apparatus. P) Pump; R) compressed air reservoir; EV) electromagnetic valve; RM) recording manometer; DK) disconnecting capsule; K_1) capsule with thin rubber membrane and contacts; K_2) membrane manometer with contacts; Cl_1-Cl_5) screw clamps; V_1) TG1 thyratron 0.1/0.3; V_2) twin triode 6H8 (half the valve is used); Re_1 and Re_2) telephonic type relay; TR) transformer; C) 4 μf; R_1) 100,000 ohm, R_2) 1 meg; R_3) 500 ohm; R_4) 1 meg; R_5) 200 ohm; R_6) 1000 ohm; R_7) 5000 ohm.

artery is fully compressed and does not affect the pressure changes in the cuff.

The essential scheme of the apparatus is shown in Fig. 1. By means of the pump P the air pressure in the reservoir R (volume about 10 l) is maintained at a constant level (250-300 mm of mercury). Through the electromagnetic valve EV, air enters a system of tubes connecting the reservoir, the sphygmomanometer cuff SC and the recording manometer RM. The rate of entry of air into the system is regulated by means of screw clamps Cl_1 and Cl_2. The clamp Cl_4, is designed to regulate the rate of leakage of air from the system. The pulse oscillations are received by a capsule K_1 with a thin rubber membrane and adjustable contacts. So that the pressure inside the cuff and its slow variations should not be transmitted directly to this capsule, it is connected with the latter through a disconnecting capsule DK, with a thicker rubber membrane, and the capsule K_1 system is supplied with a controlled leakage of air through Cl_5.

The cuff is wrapped around the arm of the subject (or the dog's thigh), and the pressure inside it is at once raised to a value known to be higher than the systolic pressure. For this purpose the clamp Cl_2 is opened, to the accessory tube leading from the reservoir to the system and by-passing the electromagnetic valve. After this has been done the clamp Cl_2 is screwed home. Thanks to the leakage of air through the clamp Cl_4 the pressure in the system begins to fall. When it reaches the level of the systolic pressure, the capsule K_1 begins to respond to the pulse oscillations, which leads to closure of its contacts. This removes the negative voltage from the grid of the thyratron V_1, which is thereby made to discharge, and the relay Re_1 which is included in its anode circuit, is set in operation.

When the contacts in K_1 are broken, a negative voltage is once more applied to the grid of the thyratron and it is extinguished (the anode circuit is fed by an alternating current). The valve V_1 acts merely as an intermediate electronic relay, permitting the use of oscillations of very small amplitude and power of the membrane of capsule K_1 for controlling the operation of the electromagnetic valve, which requires greater power. Closure of the contacts in the relay Re_1 sets in operation the relay Re_2, which directly controls the electromagnetic valve. Thus the first variation in the pulse pressure in the cuff to appear when the pressure inside the cuff is lowered leads to opening of the electromagnetic valve and to a slight increase in the pressure in the system. So that with each operation of the valve roughly the same volume of air enters the system, there is a membrane manometer K_2 with adjustable contacts, on closure of which a negative voltage is applied to the grid of the second valve V_2, and the circuit of the electromagnetic valve is broken through the relay Re_2. The contacts of the manometer are so adjusted that they always close at a certain pressure (for example 250 mm of mercury).

When the pressure inside the cuff is higher than the systolic pressure, the oscillations cease, the valve cannot open and the pressure inside the system once more begins to fall gradually until oscillations reappear. The pressure inside the cuff automatically follows behind the systolic pressure, sometimes rising and sometimes falling below its level. By controlling the rate of entry of air into the system and the rate of its escape from it, it is possible to arrange that these variations of pressure inside the cuff do not differ by more than a few mm of mercury from the level of the systolic pressure.

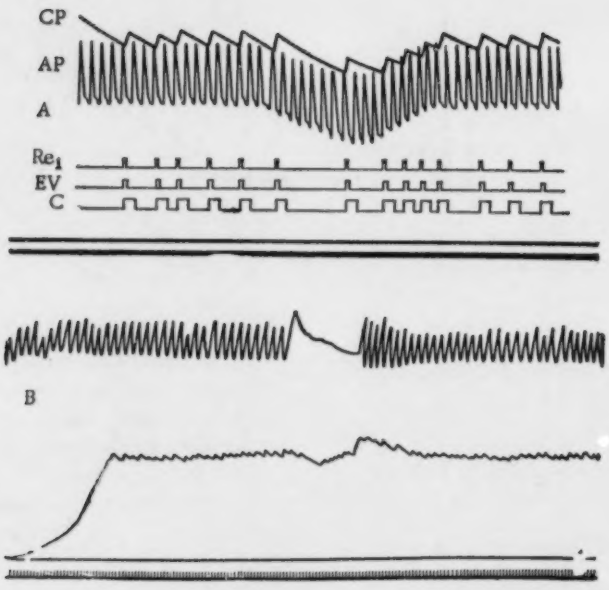

Fig. 2. Diagram illustrating the action of the apparatus (A) and an actual recording of the systolic pressure in a human subject by the method described, using a mercury manometer and an ink-recording apparatus (B); CP) pressure inside the sphygmomanometer cuff; AP) intra-arterial pressure; Re_1) moment of closure of the contacts in K_1 and operation of the relay Re_1; EV) operation of the relay R_2 and opening of the electromagnetic valve; C) time of cut-off of the valve V_2 as a result of the negative charge on the plate of the condenser connected to the grid of the valve. Significance of the curves for Fig. 2,B (from below upwards): respiration, systolic pressure, zero line, time marker (1 sec.).

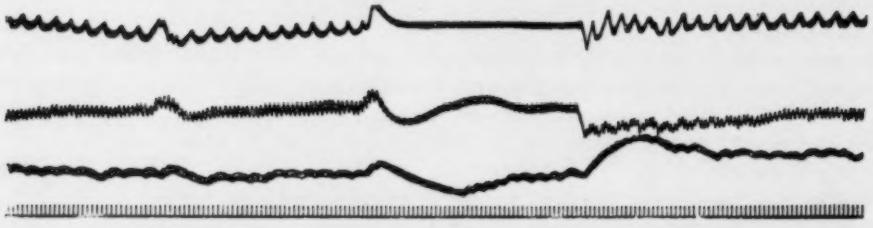

Fig. 3. Changes in the pressure during Valsalva's test (recording made with the same apparatus). The pointer of a mercury manometer was placed in front of the aperture of a photokymograph, illuminated with a parallel beam of light. Significance of the curves (from above down): respiration, finger plethysmogram, systolic pressure, time marker (1 second); the level of the time marker corresponds to 100 mm of mercury on the recording manometer.

The rate of fall of pressure inside the cuff in consequence of the escape of air must be from $^1/_3$ to $^1/_4$ of the rate of change of pressure inside the cuff at the moment of the pulse oscillation, otherwise in the course of recording of a rapidly falling pressure the gap between the contacts in K_1 will be increased and the pulse variations which appear will be unable to cause them to close. In precisely the same way the rate of increase of the

pressure in the system must not be too great, otherwise the contacts will remian closed irrespective of the presence or absence of pulse oscillations. In this way the limit of mobility of the whole system is defined, and it is able to record without any delay only relatively slow changes in the systolic pressure.

The valve V_2 is provided so that a rapid increase in the pressure inside the system at the moment of operation of the electromagnetic valve does not lead to the same closure of the contacts in K_1 as occurs during the pulse oscillations. By selection of the values of the resistors R_6 and R_7, when the contacts in K_2 are closed a negative voltage is applied to the grid of the valve of a magnitude which can break the relay Re_2. At the same time the condenser C discharges. By means of the variable resistor R_4, the rate of discharge of the condenser is regulated in such a way that closure of the contacts in K_1, due to the increased pressure inside the system at the time of opening of the valve, takes place at the moment when the negative potential of the grid was still at a sufficiently high value for operation of the relay Re_2 not to occur. In this way it is possible, by means of the resistor R_4, to regulate the length of the time interval during which the routine closure of the contactacts in K_1 does not lead to opening of the electromagnetic valve.

In Fig. 2,A is given a diagram illustrating the working of the apparatus, and in Fig. 2,B and Fig. 3 — curves obtained during recording of the blood pressure in a human subject.

Comparison of the absolute values of the pressure obtained by means of the apparatus described with those determined by Korotkov's method of auscultation showed that with a minimal gap between the contacts in K_1 the apparatus always gives slightly higher figures. The average excess, calculated from the results of 200 measurements of the arterial pressure in 6 persons, was 15 ± 10 mm of mercury. This is due to the fact that the appearance of pulse oscillations during the gradual fall of the pressure inside the cuff takes place slightly earlier than the influx of blood into the peripheral part of the limb. By increasing the gap between the contacts in K_1 or the escape of air through Cl_5, it is possible to slightly reduce the sensitivity of the capsule and to obtain accurately corresponding values for the pressure as recorded by the apparatus and by the method of auscultation. However this adjustment has to be done afresh on each new subject, which is a great nuisance. Maintenance of the pressure inside the cuff a few mm above the level of the systolic pressure is an advantage, because when making the recording, on account of the total cessation of the flow of blood in the distal portion of the limb, no overdistension of the venous system with blood takes place, and the time of recording may be greatly increased without producing any undesirable subjective sensations in the subject. The influx of blood into the peripheral portion of the limb is possible only in cases where in the course of the investigation the systolic pressure rises so rapidly that the recording system lags behind the actual changes in pressure. The possibility of overdistension of the compressed limb with blood can be reduced still further by elevation of the limb for 20-30 seconds before the start of the investigation.

The influence of movements of the muscles on the working of the apparatus is slightly diminished, because the time of opening of the electromagnetic valve is independent on the duration of closure of the contacts in K_1, provided that this does not exceed the time of discharge of the condenser C. For this reason even a large movement leads only to a single operation of the valve and to a comparatively small rise in pressure inside the apparatus. However, repeated muscular movements may considerably distort the recording, and so the investigations must be performed with the greatest possible attention to prevention of movement of the subject. This is particularly so of experiments on dogs, which must lie immobile during the experiment.

The suggested method of bloodless recording of pressure is particularly suitable for recording the changes in blood pressure casued by transient reflex and humoral factors. In clinical research this method may be used to determine the reactivity of the cardiovascular system in different tests and to investigate the action of various therapeutic measures.

SUMMARY

The author describes a new method of bloodless registration of systolic pressure in man and in dogs. It differs from other methods, based on the principle of tracing system, by the fact that the entrance of air into the sphygmomanometric cuff is controlled by pulse oscillations in the cuff and not by pulse waves in the peripheral parts of the extremity. This method has the following advantages: the work of this apparatus is independent of the local vascular reaction, there is no overdilatation of the venous system by the blood; the sensitivity to muscular motions is lower.

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A BLOODLESS METHOD OF DETERMINING THE SYSTOLIC ARTERIAL PRESSURE AND THE DEGREE OF VASODILATATION AND VASOCONSTRICTION IN WHITE RATS

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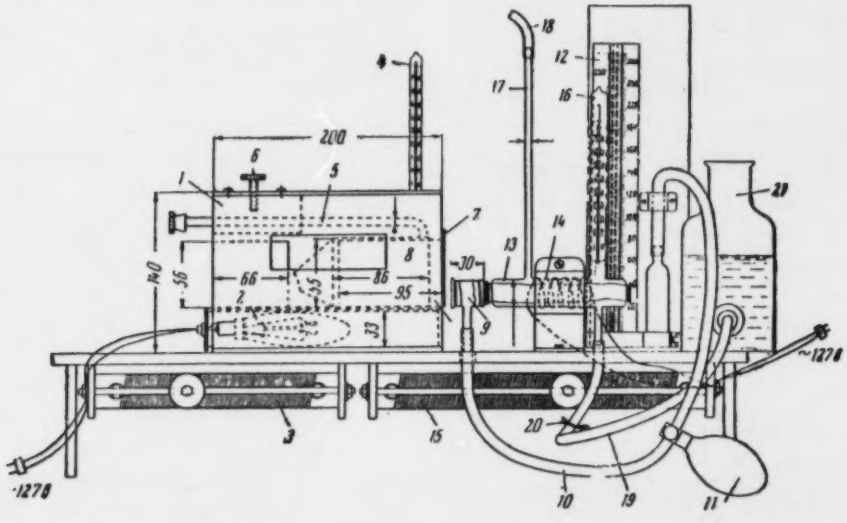
The apparatus suggested by [Williams, Harrison and Grollman (1939)] for determining the systolic arterial pressure in rats has been widely used in laboratory practice. This method has, however, the following essential faults: 1) an excessively high temperature (40-43°C) for the rats in the heated chamber (heated by an electric coil), which causes gross changes in the circulatory system, and 2) the level of the water temperature in the plethysmograph is not determined with accuracy.



Improved apparatus for the bloodless determination of the systolic arterial pressure and vascular tone in rats.

Chamber for the rat (1), electric lamp of specification 127 v, 25 w, Resk (2), rheostat (3), thermometer (4), rod for moving the chamber (5), screw for fixing the position of the chamber (6), door (7), movable part of the chamber (8). Compression chamber, diameter 18 mm (9), rubber tube (10), bulb (11), sphygmomanometer scale (12). Glass plethysmograph, 132 mm long, tube diameter 25.5 mm). Inlet diameter 18 mm, outlet diameter 9 mm (13), electric coil (14), rheostat (15), thermometer (16), water manometer (17)(internal diameter 2.5 mm), rubber connection (18). Rubber tube connecting the plethysmograph to the bottle (19). Clamp (20, water bottle (21).

*Senior Laboratory Technician I. L. Voronenkova assisted with this work.

Repeated Measurements of the Systolic Arterial Pressure and the Vasodilatation and Vasoconstriction in Healthy Rats (temperature of the chamber for the rats 30-32°C; water temperature in the plethysmograph 40-42°C)

Animal No.	Time of investigation	Time of investigation (in minutes) 1			2			3			4			5		
		pulsation	systolic arterial pressure (in mm)	dilatation (in mm)	constriction (in mm)	pulsation	systolic arterial pressure (in mm)	dilatation (in mm)	constriction (in mm)	pulsation	systolic arterial pressure (in mm)	dilatation (in mm)	constriction (in mm)	pulsation	systolic arterial pressure (in mm)	dilatation (in mm)	constriction (in mm)
1	10 hr 10 min	|	60	0.005	0.005	+	90	0.01	0.01	+	114	0.02	0.03	+	124	0.02	0.04
2	10 17	|	54	|	|	|	78	0.005	0.005	|	80	0.05	0.05	+	94	0.01	0.01
3	10 23	|	50	|	|	|	70	0.005	0.005	+	100	0.01	0.01	|	|	|	|
4	10 33	|	48	|	|	|	80	0.01	0.005	|	82	0.01	0.03	+	100	0.02	0.04
5	10 38	|	96	0.01	0.01	+	90	0.01	0.01	+	104	0.01	0.02	+	106	0.01	0.02
6	10 50	|	102	0.01	0.03	+	108	0.01	0.03	+	110	0.01	0.03	+	110	0.01	0.03
7	11 04	|	70	0.01	0.005	|	96	0.02	0.02	+	110	0.02	0.04	+	112	0.01	0.03
8	11 19	|	62	0.01	0.01	|	82	0.03	0.03	|	96	0.02	0.04	+	110	0.01	0.03
9	13 23	|	80	—	—	|	84	0.01	0.01	|	92	0.01	0.02	|	108	0.02	0.04
10	13 30	|	82	0.02	0.01	+	96	0.03	0.02	+	106	0.02	0.01	+	118	0.02	0.04

In the course of our work, at the instigation of N. I. Arinchin we have introduced the following modifications into Williams' apparatus: a more suitable double-walled chamber has been constructed; in order to increase its thermal resistance its internal surface is covered with asbestos, and an evenly distributed form of electric heating has been introduced by means of two small electric lamps (25-30 w), the emission of heat from their filaments being controlled by a rheostat. This enables the temperature of the chamber to be kept constant (30-32°C); the metal plug separating the plethysmograph from the compression cuff has been removed as superfluous. A thermometer has been introduced into the plethysmograph for recording the water temperature. Electric heating has been introduced into the plethysmograph, as described by S. K. Krasnov [4], and its temperature is regulated by a rheostat. This allows the water temperature in the plethysmograph to be maintained at a constant level (40-42°C). The manometer has been transferred from the end portion of the plethysmograph to the point on the apparatus corresponding to the beginning of the animal's tail, where larger vascular trunks are to be found. The capillary tube is replaced by a manometer with an internal diameter of 2-2½ mm. Instead of injecting water into the plethysmograph with a syringe, a siphon system has been introduced.

So that the tail can be introduced more conveniently into the plethysmograph, the rubber tube which is fixed to the free end of the manometer is clamped and the bottle of water is lowered below the level of the plethysmograph. Enlarging the lumen of the cuff allows the tail to be introduced freely into the plethysmograph. The modifications which have been carried out enable the experiments to be conducted at a constant temperature, which is essential, and they make the apparatus much easier to use. The details of the apparatus are shown in the Figure.

The constant temperature conditions in the apparatus allows observations to be made on the level of the systolic arterial pressure for 15-30 minutes.

The value of the systolic arterial pressure is determined numerically by the level of the mercury in the Riva-Rocci apparatus in the period of decompression, at the beginning of the rise of the fluid in the plethysmograph. The results of our investigations of the level of the systolic arterial pressure in healthy white rats are shown in the table.

These findings enable the trends of the systolic arterial pressure in healthy rats to be demonstrated under given temperature conditions. As a result of heating the rats, the level of their blood pressure rises. The first reading of the blood pressure approximates the natural value most closely, and may be compared with the initial values obtained from the first measurements under different experimental conditions. The tendency of the blood pressure to rise provides evidence of the state of the circulation when the body temperature rises. These readings may also be compared with those obtained during the action of various factors on the cardiovascular system, and thereby give an indication of the hyper-or hypotensive effect of different drugs. The average value of all the readings may also be taken. The appearance of a pulsating flow of blood during the investigation may indicate an increase in the propulsive power of the heart and, possibly, enlargement of the lumen of the caudal vessels.

Besides the more accurate bloodless determination of the level of the blood pressure, it is possible with this modified method to estimate at the same time the vascular tone by N. I. Arinchin's method [1, 2, 3]. The state of the vascular tone is expressed by the degree of vasodilatation and vasoconstriction of the caudal vessels, which is determined by the magnitude of the rise of the column of water in the manometer of the plethysmograph in the period of compression and decompression. Thanks to the introduction into the plethysmograph of a graduated manometric tube, 1 ml in volume and with divisions of 0.01 ml, the dilatation of the caudal vessels and their subsequent constriction can be measured with an accuracy of 0.01 ml by the rise and fall of the fluid level in the plethysmograph tube.

From the experience we have gained, the following method of investigation of the blood pressure and the vascular tone in white rats appears to be the most rational.

The chamber for the rat is heated to 25°C, after which the rat is placed inside and the electric heater switched off. After a few minutes the temperature of the chamber rises to 27°C and remains within these limits, with only slight variations for a period of many hours. Introduction of the rat into the chamber heated only to 21°C also causes the temperature of the chamber to rise gradually to 27°C. If the rat is placed in the chamber heated to 40-42°C, as recommended by Williams, the temperature of the chamber falls by 3-4°C. Thus by the method which we recommend it is not the chamber that heats the rat but the rat that heats the chamber to the temperature of its own body. Accordingly it is possible at the same time to study the process of thermoregulation (the intensity of heat emission, and the heat production, by measurement of the body temperature) in white rats under various experimental conditions. In the normal course of this work it is necessary to keep the rats at room temperature (20°C) outside the period of the investigation as well.

The frequency of measurement is of great importance. If the blood pressure is measured every minute for a long time, a reflex strengthening of the action of the heart takes place, leading to a rise in the blood pressure, while at the same time there is increased dilatation, and especially constriction of the vessels. It is therefore necessary to carry out the measurements at intervals of 3-5 minutes, and if the investigations are to last several hours this interval may be lengthened.

It is also important, if the tail is not removed, to release it from compression by the plethysmograph cuff, otherwise this causes distress to the animal, which alters its cardiac activity. It is therefore necessary, in the intervals between the individual measurements, to restore the normal blood supply to the caudal vessels without fail.

If these conditions are observed, the blood pressure is kept within limits of 80-90-110 mm of mercury in the course of observation for 4-6 hours, and the vasodilatation and vasoconstriction measure 0.02-0.04 mm of water. This is in agreement with the experimental results of Proskauer, Neumann and Graef [6]. Hence the values obtained by Williams' method vary significantly from the normal level of the blood pressure in white rats.

SUMMARY

The authors improved the Williams-Grollman-Harrison apparatus. A more convenient double-walled chamber was devised, covered by asbestos for maintaining the constant temperature. The temperature regimen of the chamber was changed. Instead of 40-42°C the investigations are carried out at 30-31°C which removes the thermal dyspnea and the thermal shock in experimental animals. The plethysmograph is filled with water by the syphon method. The temperature of the water is determined by the mounted thermometer and is regulated with the aid of a rheostat. The manometer is transferred from the caudal part of plethysmograph to its proximal part where

the large arteries of the tail pulsate. This gives more precise recordings. This apparatus permits determination of the vasoconstriction in the tail by N. I. Arinchin's method. The rise and fall of the manometric fluid level during the period of decompression is taken into consideration.

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THE USE OF LEAD NITRATE FOR DETECTION OF THE INTRACELLULAR APPARATUS RETICULAR

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We propose a modification of Cajal's method, by substituting lead nitrate for uranium nitrate. The material should be treated as follows:

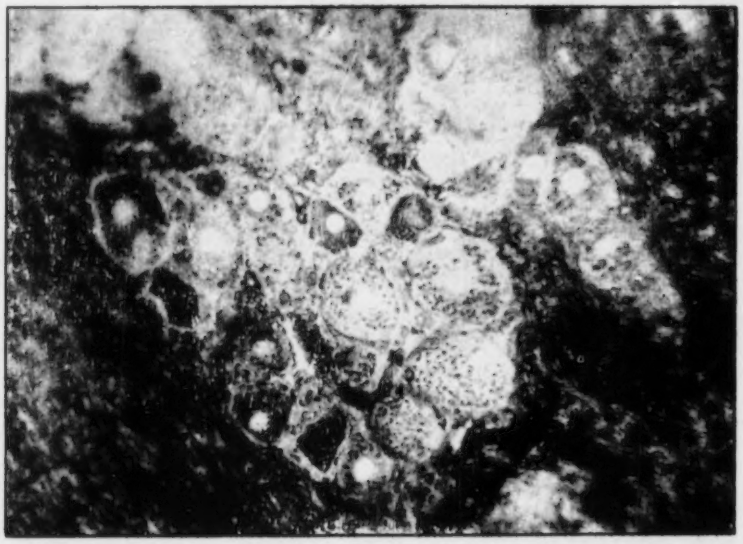

Fig. 1. Section of the spinal ganglion of a cat. Stained by a modification of Ramón y Cajal's method to detect the Golgi apparatus. Magnification: ocular 7x, objective 40x.

1). Fixation for 10 hours in the mixture: lead nitrate – 1 g, neutral formalin – 15 ml, distilled water – 100 ml.

2). After rapid rinsing in distilled water the pieces of material are kept for 24 hours in a 1.5% solution of silver nitrate.

3). The specimens are placed for 2-5 hours in a mixture of hydroquinone (2 g), formalin (10 ml) and distilled water (100 ml) to which has been added anhydrous sodium sulfate, a grain at a time until it dissolves, until the mixture acquires the color of tea.

Fig. 2. Section of the vagus nerve ganglion of a cat. Stained by a modification of Ramón y Cajal's method to detect the Golgi apparatus. Magnification: ocular 7x, objective 40x.

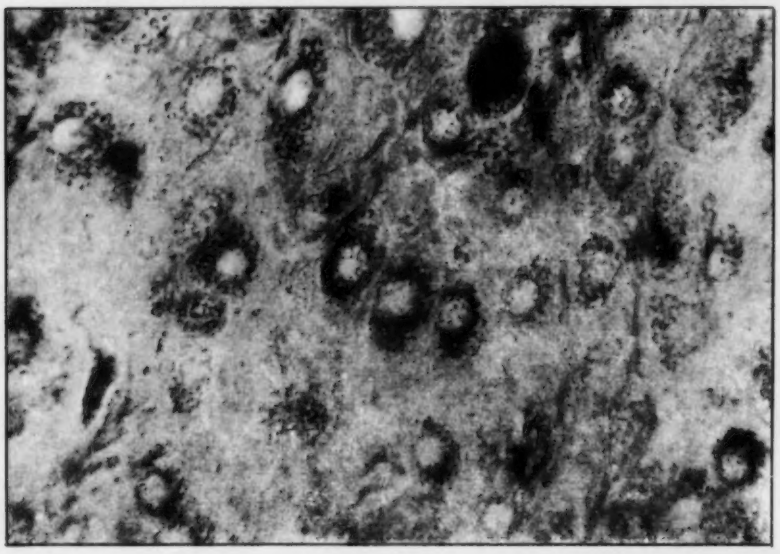

Fig. 3. Section of the cranial cervical ganglion of a cat. Stained by a modification of Ramón y Cajal's method to detect the Golgi apparatus. Magnification: ocular 7x, objective 40x.

4). The specimens are rapidly rinsed in distilled water, taken up through increasing strengths of alcohol and embedded in paraffin wax.

In this work we selected small fragments of organs (up to 0.5 cm in thickness). This enabled the material to be taken through alcohol quickly: for 1 hour in the 45° and 70°, 3 hours in the 96° and 3-6 hours in the absolute alcohol.

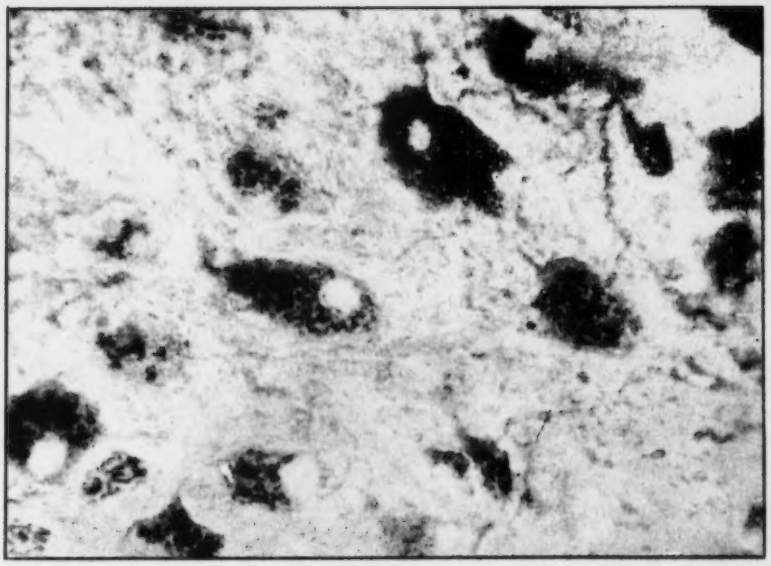

Fig. 4. Section of the semilunar ganglion of a cow. Stained by a modification of Ramón y Cajal's method to detect the Golgi apparatus. Magnification: ocular 7x, objective 40x.

By using the method described, we obtained good results in examinations of the spinal ganglia (Fig. 1), the ganglion nodosum (Fig. 2) and the cranial cervical ganglion (Fig. 3) of cats and the semilunar (Fig. 4) and the stellate ganglia of cattle. The Golgi apparatus stains a black color.

In sections of the spinal ganglia differences are clearly seen in the size, intensity of staining and the character of the color hues of the nerve cells (Fig. 1). As a rule the protoplasm of the larger cells is stained less intensely. In other ganglia which we investigated, there was little difference between the nerve cells as regards the intensity of staining.

SUMMARY

As a result of experiments it was established that lead nitrate may be employed for the detection of the internal reticular apparatus. Together with the neutral formalin, lead nitrate is included into the content of the fixating solution. The preparation is kept in the silver nitrate solution and, finally, in the mixture of hydroquinone, sodium sultate and formalin. Then the material is quickly passed through the alcohols and is embedded in paraffin. The whole process takes about 5 days. Following this treatment Golgi apparatus are clearly seen in the nerve cells of various ganglia in mammals, epithelial cells of the small intestine, cells of the corpus luteum and oocytes.

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SOME PRACTICAL OBSERVATIONS ON THE BIELSCHOWSKY – GROS IMPREGNATION METHOD

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During research into the peripheral innervation of organs we have succeeded in introducing a number of modifications into the Bielschowsky-Gros silver method, which we mainly used. This paper is devoted to a description of these modifications, which enable some of the inherent faults of this method to be corrected.

1). Obtaining, fixing and preservation of the material. For impregnation it is best to use fresh material obtained soon after death, but if human tissue is to be examined this can be done during the first 48 hours provided that proper conditions of preservation are provided. Specimens of organs (or tissues) taken for examination must be well rinsed free of blood and from water-soluble proteins. This is best achieved by washing in a 5% solution of magnesium sulfate for 5-40 minutes, depending on the thickness and firmness of the specimen. After being washed in this solution, the material is fixed in 15-20% neutral formalin. Fixation continues for not less than 7 days. The material may be preserved in neutral formalin for months without losing its ability to undergo impregnation, especially if 10-25 g of chalk is placed in the bottom of the vessel containing the neutral formalin.

If after prolonged keeping the quality of impregnation of the specimens deteriorates, they may be stained again, but this time a 20% solution of magnesium sulfate must be used, and the specimens must be placed in freshly prepared 12% neutral formalin.

The superficial layers of the fixed material may impregnate poorly, and so it is best to cut them away before impregnation. When the superficial layers are themselves of interest to the investigation and may not therefore be removed, the material is fixed in two stages: at first for 2-3 days in a 6% solution of neutral formalin, and for the remainder of the time in 15% formalin. The neutral formalin is prepared by standing on purified chalk, on magnesium carbonate or calcined magnesia, or by titration with caustic alkali.

2). Impregnation. Immediately before sections are cut, the pieces of fixed material are washed (for a sample 1 cm^3) in tap water for 3-5 minutes, after which they are transferred to a Petri dish with distilled water, and quickly cut on a freezing microtome. The sections are collected in the same dish, without changing the distilled water in it, so that traces of formalin remain in the sections. This is one of the essential conditions for successful impregnation.

In case of unsuccessful impregnation, the sections or pieces of tissue which have been washed excessively free from formalin may be replaced for 1-3 hours or overnight in 5% neutral formalin. Before impregnation such material is washed for 2-5 minutes in the same sample of distilled water.

The washed sections are placed in a 10% solution of silver nitrate for 5-30 minutes until they acquire a yellowish color. When they are taken out of the distilled water they are lightly dried by wrapping in filter paper.

After their first treatment with silver nitrate solution, the sections are passed through the same sample of 20% acid formalin, which it is advisable to heat to 25-30°C. This operation is carried out as quickly as possible (not longer than 5 seconds). The sections are placed on a glass rod and immersed in formalin (in one dish) to wash each section on all sides with the solution, and they are then just as quickly and carefully dried free from formalin between layers of filter paper. Light expression of the formalin by pressure on the filter paper may bring about removal of formalin from the sections. If the nature of the material does not permit the use of this method of removal of formalin from the sections, they may rapidly be taken through distilled water. Freeing the impregnated sections from formalin by one of the methods indicated is essential, otherwise they will momentarily blacken in the ammoniacal silver solution, which is one of the main causes of failure of impregnation.

After the sections have been freed from formalin they are placed in the ammoniacal silver, prepared according to Gros's formula. The sections may be kept in the ammoniacal silver solution without harm to the subsequent treatment for 5 minutes to 1-2 hours. After immersion in the solution, the sections rapidly acquire a brownish hue. In the first place test sections must be stained; if these become brown in patches, then 2-5 drops of ammonia must be added to the ammoniacal silver solution.

The metallic silver in the nerve cells is readily reduced in boiled and cooled water (15-25 ml) with the addition of 1-2 drops of 20% acid formalin. It is best to take these 1-2 drops of formalin not from pure (unused) formalin, but from the dish in which the sections were immersed after the first silver bath. Impregnation proceeds evenly in this weak formalin solution; the sections acquire a brown tone with an orange hue. If impregnation proceeds too quickly or too slowly it is necessary to vary the number of drops of formalin added to the water; where impregnation is extremely rapid, 1 drop of formalin per Petri dish (15-25 ml) of boiled water is sufficient, and when it is slow, drops of formalin may be added up to a volume of 0.1-1 ml. As a control, isolated sections are taken out of the dish at different times, and placed on a glass slide for examination under the microscope. On the appearance of a clear pattern of nerve cells (which takes place quite quickly), the reduction must be brought to an end without delay and the sections are transferred to cooled, boiled water. Should the sections continue to darken, glacial acetic acid is added to the water at the rate of 1 drop per 25 ml. After washing (for 1-2 hours) in acidified water, the sections are rinsed in distilled water and taken up through alcohol for clearing and mounting in balsam.

If the sections have been kept too long in the reducing formalin solution, so that they have acquired a dark brown tone, they may be placed in a 1% solution of ammonia and allowed to remain there until lighter tones have been produced (but not for longer then 1 second), after which they are quickly washed in distilled water and transferred to alcohol.

If impregnation is unsuccessful on account of an inadequate length of stay of the sections in the silver solutions, and they appear pale yellow in tone with no clear pattern of the nerve cells, these sections may be placed in distilled water for a few minutes and then transferred to the first (10%) solution of silver nitrate, after which the procedure is repeated as described above.

When the sections are to be counterstained with aniline dyes, gold impregnation is required as a preliminary step. The bath for impregnation with gold is prepared as follows. To 10-20 ml of distilled water is added 5-10 drops of 1% gold chloride, and at the bottom of the dish is added 2-3 g of purified chalk; this is stirred with a glass rod, the particles of chalk allowed to settle, and the purified gold chloride solution is then poured into a clean dish. The sections are placed in this dish after they have been washed for a few seconds in distilled water until they turn grey, and they are then quickly transferred to 3% hyposulfite (chemically pure) for 5-10 minutes. After the sections have been thoroughly rinsed free from hyposulfite they are counterstained with aniline dyes, dehydrated with alcohol, cleared and mounted in balsam. If no chalk is used, the gold chloride causes severe decolorization of the sections, so that the pattern of the neurofibrils may completely disappear.

Our modification of the Bielschowsky -Gros method is simpler and more convenient. It is possible to stain a large number of sections (hundreds) simultaneously, thereby resulting in considerable economy of time and of expensive chemicals. Furthermore serial treatment of the material is possible, for all the sections without exception are impregnated to the same desired color — from dark yellow to dark brown; under these circumstances the neurofibrillar apparatus of the nerve formations acquires a black tone.

SUMMARY

The author suggests a simplified modification of the method of impregnation for staining the peripheral nervous system and the nerve endings. The tecnique of impregnation is as follows: the specimen is washed in magnesium sulphate solution for 5 to 40 minutes; it is fixed and kept in 15% neutral formalin; then it is washed and sections are prepared; next the sections are impregnated by 10% $AgNO_3$ and are transferred into 20% acid formalin and then into distilled water (second) and are thouroughly dried between pieces of filter paper (important), then it is passed into 10% $AgNO_3$ where it is kept for 5-10 minutes; metallic silver is reduced (under microscopic control) in cups with boiled and cooled water with an addition of 1 to 5 or more drops of 20% acid formalin (a test is to be made); then it is rinsed in distilled water and dehydrated in alconol of increasing concentration after which the sections are embedded in the fir tree balsam. Sections may be stained with gold (before dehydration in alcohols). 1% gold chloride should be mixed with pure calcium chloride (3:1) and 1-4 drops of this solution per cup of distilled water should be taken for gold impregnation of the preparation, then it may be additionally stained by 5% hyposulphite (not necessarily).

ABBREVIATIONS MOST FREQUENTLY ENCOUNTERED IN RUSSIAN BIO-SCIENCES LITERATURE

Abbreviation (Transliterated)	Significance
AMN SSSR	Academy of Medical Sciences, USSR
AN SSSR	Academy of Sciences, USSR
BIN	Biological Institute, Botanical Institute
FTI	Institute of Physiotherapy
GONTI	State United Sci-Tech Press
GOST	All Union State Standard
GRRRI	State Roentgenology, Radiology, and Cancer Institute
GTTI	State Technical and Theoretical Literature Press
GU	State University
I Kh N	Scientific Research Institute of Surgical Neuropathology
IL (IIL)	Foreign Literature Press
IONKh	Inst. Gen. and Inorganic Chemistry (N. S. Kurnakov)
IP	Soil Science Inst. (Acad. Sci. USSR)
ISN (Izd. Sov. Nauk)	Soviet Science Press
Izd.	Press
LEM	Laboratory for experimental morphogenesis
LENDVI	Leningrad Inst. of Dermatology and Venereology
LEO	Laboratory of Experimental Zoology
LIKhT	Leningrad Surgical Institute for Tuberculosis and Bone and Joint Diseases
LIPZ	Leningrad Inst. for Study of Occupational Diseases
LIPK	Leningrad Blood Transfusion Institute
Medgiz	State Medical Literature Press
MOPISh	Moscow Society of Apiculture and Sericulture
MVI	Moscow Veterinary Institute
MZdrav	Ministry of Health
MZI	Moscow Zootechnical Institute
LOKhO	Leningrad Society of Orthopedic Surgeons
NIIZ	Scientific Research Institute of Zoology
NINKhI	Scientific Research Institute of Neurosurgery
NIU	Scientific Institute for Fertilizers
NIUIF	Scientific Research Institute of Fertilizers and Insecticides
NIVI	Veterinary Scientific Research Institute
ONTI	United Sci.-Tech. Press
OTI	Division of Technical Information
RBO	Russian Botanical Society
ROP	Russian Society of Pathologists
SANIIRI	Central Asia Scientific Research Institute of Irrigation
SANIISh	Central Asia Scientific Research Institute of Sericulture
TsNII	All-Union Central Scientific Research Institute
TsNTL	Central Scientific and Technical Laboratory
VASKhNIL	All-Union Academy of Agricultural Sciences
VIG	All-Union Institute of Helminthology
VIEM	All-Union Institute of Experimental Medicine
VIR	All-Union Institute of Plant Cultivation
VIUAA	All-Union Institute of Fertilizers, Soil Science, and Agricultural Engineering
VIZR	All-Union Institute of Medical and Pharmaceutical Herbs
VNIRO	All-Union Scientific Institute of Fishing and Oceanography
ZIN	Zoological Inst. (Acad. Sci. USSR)

Note: Abbreviations not on this list and not explained in the translation have been transliterated, no further information about their significance being available to us. - Publisher.

RUSSIAN JOURNALS FREQUENTLY CITED
[Biological Sciences]

Abbreviation*	Journal*	Translation
Agrobiol.	Agrobiologiia	Agrobiology
Akusherstvo i Ginekol.	Akusherstvo i Ginekologiia	Obstetrics and Gynecology
Antibiotiki	Antibiotiki	Antibiotics
Aptechnoe Delo	Aptechnoe Delo	Pharmaceutical Transactions
Arkh. Anat. Gistol. i Embriol.	Arkhiv Anatomii Gistologii i Embriologii	Archives of Anatomy, Histology, and Embryology
Arkh. Biol. Nauk SSSR	Arkhiv Biologicheskikh Nauk SSSR	Archives of Biological Science USSR
Arkh. Patol.	Arkhiv Patologii	Archives of Pathology
Biofizika	Biofizika	Biophysics
Biokhimiia	Biokhimiia	Biochemistry
Biokhim. Plodov i Ovoshchei	Biokhimiia Plodov i Ovoshchei	Biochemistry of Fruits and Vegetables
Bot. Zhur.	Botanicheskii Zhurnal	Journal of Botany
Biull. Eksptl. Biol. i Med.	Biulleten Eksperimentalnoi Biologii i Meditsiny	Bulletin of Experimental Biology and Medicine
Biull. Moskov. Obshchestva Ispytatelei Prirody, Otdel Biol.	Biulleten Moskovskogo Obshchestva Ispytatelei Prirody, Otdel Biologicheskii	Bulletin of the Moscow Naturalists Society, Division of Biology
Doklady Akad. Nauk SSSR	Doklady Akademii Nauk SSSR	Proceedings of the Academy of Sciences USSR
Eksptl. Khirurg.	Eksperimentalnaia Khirurgiia	Experimental Surgery
Farmakol. i Toksikol.	Farmakologiia i Toksikologiia	Pharmacology and Toxicology
Farmatsiia	Farmatsiia	Pharmacy
Fiziol. Rastenii	Fiziologiia Rastenii	Plant Physiology
Fiziol. Zhur. SSSR	Fiziologicheskii Zhurnal SSSR im. I. M. Sechenova	I. M. Sechenova Physiology Journal USSR
Gigiena i Sanit.	Gigiena i Sanitariia	Hygiene and Sanitation
Izvest. Akad. Nauk SSSR, Ser. Biol.	Izvestiia Akademii Nauk SSSR, Seriia Biologicheskaia	Bulletin of the Academy of Sciences USSR, Biology Series
Izvest. Tikhookeanskogo N. I. Inst. Rybnogo Khoz. i Okeanog.	Investiia Tikhookeanskogo N. I. Instituta Rybnogo Khoziaistva i Okeanografii	Bulletin of the Pacific Ocean Scientific Institute of Fisheries and Oceanography
Khirurgiia	Khirurgiia	Surgery
Klin. Med.	Klinicheskaia Meditsina	Clinical Medicine
Lab. Delo	Laboratornoe Delo (po Voprosam Meditsiny)	Laboratory Work (on Medical Problems)
Med. Parazitol.	Meditsinskaia Parazitologiia i Parazitarnye Bolezni	Medical Parasitology and Parasitic Diseases
Med. Radiol.	Meditsinskaia Radiologiia	Medical Radiology
Med. Zhur. Ukrain.	Medichnii Zhurnal Ukrainskii	Ukrainian Medical Journal
Mikrobiologiia	Mikrobiologiia	Microbiology
Mikrobiol. Zhur.	Mikrobiologichnii Zhurnal	Microbiology Journal
Nevropatol., Psikhiat. i Psikhogig.	Nevropatologiia, Psikhiatriia i Psikhogigiena	Neuropathology, Psychiatry and Psychohygiene
Ortoped., Travmatol. i Protez.	Ortopediia, Travmatologiia i Protezirovanie	Orthopedics, Traumatology and Prosthetics
Parazitol. Sbornik	Parazitologicheskii Sbornik	Parasitology Collection
Pediatriia	Pediatriia	Pediatrics
Pochvovedenie	Pochvovedenie	Soil Science
Priroda	Priroda	Nature
Problemy Endokrinol. i Gormonoterap.	Problemy Endokrinologii i Gormonoterapii	Problems of Endocrinology and Hormone Therapy
Problemy Gematol.	Problemy Gematologii i Perelivaniia Krovi	Problems of Hematology and Blood Transfusion
Problemy Tuberk.	Problemy Tuberkuleza	Problems of Tuberculosis
Sovet. Med.	Sovetskaia Meditsina	Soviet Medicine
Sovet. Vrachebny Zhur.	Sovetskii Vrachebnyi Zhurnal	Soviet Physicians Journals
Stomatologiia	Stomatologiia	Stomatology
Terap. Arkh.	Terapevticheskii Arkhiv	Therapeutic Archives
Trudy Gelmint. Lab.	Trudy Gelmintologicheskoi Laboratoriia	Transactions of the Helminthology Laboratory
Trudy Inst. Genet.	Trudy Instituta Genetiki	Transactions of the Institute of Genetics

* LIBRARY OF CONGRESS TRANSLITERATION SYSTEM.

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Abbreviation	Journal	Translation
Trudy Inst. Gidrobiol.	Trudy Instituta Gidrobiologiia	Transactions of the Institute of Hydrobiology
Trudy Inst. Mikrobiol.	Trudy Instituta Mikrobiologiia	Transactions of the Institute of Microbiology
Trudy Inst. Okean.	Trudy Instituta Okeanologiia, Akademii Nauk SSSR	Transactions of the Institute of Oceanology, Academy of Sciences, USSR
Trudy Leningrad Obshchestva Estestvoisp.	Trudy Leningrad Obshchestva Estestvoispytatelei	Transactions of the Leningrad Society of Naturalists
Trudy Vsesoiuz. Gidrobiol. Obshchestva	Trudy Vsesoiuznogo Gidrobiologicheskogo Obshchestva	Transactions of the All-Union Hydrobiological Society
Trudy Vsesoiuz. Inst. Ekspti. Med.	Trudy Vsesoiuznogo Instituta Eksperimentalnoi Meditsiny	Transactions of the All-Union Institute of Experimental Medicine
Ukrain. Biokhim. Zhur.	Ukrainskii Biokhimichnii Zhurnal	Ukrainian Biochemical Journal
Urologiia	Urologiia	Urology
Uspekhi Biokhimiia	Uspekhi Biokhimiia	Progress in Biochemistry
Uspekhi Sovremennoi Biol.	Uspekhi Sovremennoi Biologiia	Progress in Contemporary Biology
Vestnik Akad. Med. Nauk SSSR	Vestnik Akademii Meditsinskikh Nauk SSSR	Bulletin of the Academy of Medical Science USSR
Vestnik Khirurg. im. Grekova	Vestnik Khirurgii imeni Grekova	Grekov Bulletin of Surgery
Vestnik Leningrad. Univ. Ser. Biol.	Vestnik Leningradskogo Universiteta, Seriia Biologii	Journal of the Leningrad Univ., Biology Series
Vestnik Moskov. Univ., Ser. Biol. i Pochvov.	Vestnik Moskovskogo Universiteta, Seriia Biologii i Pochvovedeniia	Bulletin of the Moscow University, Biology and Soil Science Series
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